

THE ROLE OF LITHIUM IN THE TREATMENT OF BIPOLAR DISORDER:
IDENTIFYING CLINICAL AND GENETIC PREDICTORS OF RESPONSE TO
LITHIUM TREATMENT

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Abstract

Lithium is one of the most effective treatments for bipolar disorder (BD) and is typically recommended as first-line therapy. However, the efficacy of lithium varies widely from person to person, with about 30% of patients showing partial response and another 25% no response. As a result, it would be beneficial to identify factors which predict who may respond better than others so treatment could be targeted to the appropriate patients. A growing body of evidence suggests that demographic, clinical, and genetic factors may play a role in determining the variability in treatment response. These studies, however, have typically been retrospective or were not specifically designed to examine predictors of response, and the results have been inconsistent.

This thesis examines the role of lithium in the treatment of BD and seeks to identify clinical and genetic predictors that may be used to help guide personalized treatment decisions about which patients would most benefit from treatment. The first contribution

of the thesis is a characterization of trends in lithium utilization for the treatment of BD in the U.S. over the past twenty years. To analyze the changing patterns in the management of BD, we use data from the National Ambulatory Medical Care Survey and examined trends in prescriptions of lithium along with other medication categories in visits for BD.

The second contribution of the thesis is an evaluation of clinical predictors associated with response to lithium treatment over time. Using prospective treatment data from the Pharmacogenomics of Bipolar Disorder (PGBD) study, we sought to identify clinical predictors of lithium response in an attempt to determine the clinical characteristics of lithium responders and non-responders prior to initiating treatment.

The third contribution of the thesis is to examine genetic predictors of response to lithium. Using genetic data from the PGBD and reference expression quantitative trait loci (eQTL) data derived from brain samples from the Lieber Institute for Brain Development, we tested for polygenic risk scores as predictors of lithium response, and we used a novel application of the transcriptome-wide association (TWAS) approach to identify specific genes that are associated with lithium response.

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Chapter 1

Introduction

Bipolar disorder (BD) is a severe and often disabling psychiatric disorder that affects approximately 1% to 4.4% [1, 2] of the world's population. BD is classically described as having a course of illness that alternates between extreme mood states of mania and depression with episodic relapses and more longstanding disability [3]. BD is a major cause of hospitalizations and health care expenditures, and is also associated with an increased risk of suicide [4], leading to the critical issue of bipolar treatment.

Lithium is one of the most effective treatments for BD [5, 6], and it is typically recommended as a first-line therapy [7]. However, the efficacy of lithium can vary widely from person to person, with about 30% fully responding (Alda, 2017), while about 30% show only partial response and another 25% no response [8]. Moreover, lithium is associated with serious side-effects [9], and it is recommended that lithium levels are

regularly monitored during care to avoid lithium toxicity, which can be life-threatening in rare cases. Concerns about these side-effects and the presumed challenge in managing patients while on lithium have led clinicians to increasingly prescribe other anticonvulsants and/or second-generation antipsychotics which have demonstrated mood-stabilizing effects [10-14], even though it is unclear if they are as effective as the relatively cheaper lithium. As a result, it would be beneficial to identify factors which predict who may respond better than others so treatment with lithium could be targeted to the appropriate patients.

A growing body of evidence suggests that demographic, clinical [15], and genetic [16] factors may play a role in determining the variability in response to lithium treatment. There are numerous previous studies of demographic and clinical predictors of lithium response, the results of which have been summarized in several recent reviews [17-19]. There is also evidence from family studies that genetic factors may be related to response as well [20], and several genome-wide association studies (GWAS) have been carried out to identify specific associated genetic variants [8, 21, 22]. These studies have typically been retrospective or were not specifically designed to examine predictors of response, and the results have been inconsistent.

To advance our understanding of factors that predict lithium response and contribute to the goal of precision medicine for BD, I carried out the following specific aims:

Aim 1: To characterize trends in lithium utilization for the treatment of BD in the U.S. over the past twenty years. In this aim, I used data from the National Ambulatory Medical Care Survey (NAMCS), a national survey of office-based encounters covering the use of ambulatory medical care services in the U.S. [23], to examine changing trends in the

prescription of lithium, mood stabilizing anticonvulsants, second generation antipsychotics, and antidepressants among patients with BD. The goal was to gain a better understanding of how medication treatment strategies for BD have changed over the past two decades, and to confirm the observation that lithium use has been declining in favor of other psychotropic medications, despite the fact that lithium is recommended as a first-line therapy.

Aim 2: To evaluate clinical predictors associated with response to lithium treatment over time. In this aim, I used data from the Pharmacogenetics of Bipolar Disorder (PGBD) trial, an eleven site prospective trial of lithium treatment in BD [20] to investigate the association of demographic and clinical features with acute and long-term prospective response. The goal is to develop prediction models with clinical variables and test their ability to predict response over two years of clinical follow-up in the PGBD trial.

Aim 3: To evaluate genetic predictors of lithium response. In this aim, I first used genome-wide SNP genotype data on the PGBD samples, to test lead SNPs that have been implicated by prior GWAS of lithium treatment in BD. Then, I used a novel transcriptome-wide association (TWAS) approach to evaluate the association of response with specific genes and gene sets. For this, I used RNA-sequencing data on post-mortem brain samples from the Lieber Institute for Brain Development to impute into the PGBD samples gene expression levels in two brain regions of the limbic system thought to be relevant to BD, the amygdala and sub-genual anterior cingulate cortex. I conducted a TWAS to test if imputed expression levels of individual genes (or specific gene sets targeted by lithium) in these two brain regions are associated with response to lithium treatment over time. A TWAS has several advantages over traditional GWAS because it conducts tests that are

more closely related to biological mechanisms and it reduces the multiple testing burden. Finally, I tested the association of lithium response with polygenic risk scores for several related psychiatric disorders that may influence the efficacy of treatment.

The overall goal of this project is to advance our understanding of the mechanisms underlying the action of lithium and to identify clinical and genetic predictors of lithium response among patients with BD. This work is significant because it will facilitate the advent of precision guided treatment for BD by identifying those patients who would most benefit from lithium and thereby mitigate the lengthy process of trial and error to find the right treatments that often occurs for patients with this devastating disorder.

Chapter 2

Literature Review

2.1 Lithium as first-line treatment for bipolar disorder (BD)

First introduced by John Cade in 1949, the modern use of lithium for treatment of BD has been widely studied since. Findings from these studies have been at times controversial, but the evidence for the efficacy of lithium in acute mania and maintenance treatment is now well established. In a meta-analysis of five randomized controlled trials of BD patients comparing prophylactic lithium therapy with placebo, lithium was found to be more effective than placebo in preventing recurrence of illness, with 60% subjects in the lithium group remaining well over a 1-to-2-year period in comparison to 40% in placebo [5]. Subsequently, another meta-analysis of six studies of lithium for treating acute mania found that 48% of patients responded to lithium compared with 31% in placebo [24].

While these seminal reviews unequivocally demonstrate the efficacy of lithium for both acute and maintenance treatment of BD, it is acknowledged that the efficacy of lithium

varies widely, with about 30% of treated patients showing only partial response and another 25% having no response [8], although there are on-going efforts to further define lithium response [25]. In addition, lithium use is associated with certain serious side effects including reduced urinary concentrating ability, hypothyroidism, and hyperparathyroidism [26], requiring careful clinical management due to its narrow therapeutic index [27].

2.2 Other medications for BD treatment

Lithium's varying efficacy and side effects have led to the increasing use of other medications that also have demonstrated efficacy in BD, including certain anticonvulsants and second-generation antipsychotics (SGAs) [10, 11, 28]. Some of the more commonly used anticonvulsants for BD include valproic acid/divalproex sodium, lamotrigine, and carbamazepine, while some of the more commonly used SGAs include quetiapine, aripiprazole, and risperidone. However, the long-term effects of newer medications used to treat BD are still not clear and anticonvulsants may not necessarily work better than lithium in preventing the recurrence of mood episodes or in reducing the overall risk of suicide [6, 29]. Furthermore, long-term randomized controlled trials are needed to uphold SGAs' efficacy and ability to improve patients' psychosocial functioning and quality of life [28, 30].

Even though lithium is typically recommended as a first-line therapy for BD, its use has been eclipsed in recent years by these other mood-stabilizing anticonvulsants and second-generation antipsychotics. This trend has been documented by several recent studies in European countries [31-34]. In the US, trends in prescriptions of lithium and

other medications for treatment of BD have been examined through the 1990s and early 2000s using data from the National Ambulatory Medical Care Survey (NAMCS) and Kaiser Permanente, Northern California health system [35-37]. However, there is little national level data on trends since 2000. Data from a single tertiary care clinic at Stanford University between 2000 and 2011 suggested a decrease in lithium usage [38]. However, it is unclear whether this trend is generalizable beyond the one clinic. In Chapter 3, I examine trends in prescriptions of lithium and other medications for BD in a representative sample across the US over a 20-year period from 1996-2015.

2.3 Predictors of clinical response to lithium treatment

There is continued interest in identifying predictors of response to lithium treatment before treatment starts. The ultimate goal of precision medicine (also referred to as individualized or personalized medicine) is to avoid the typical trial and error process of finding the right medication for a particular patient during which time the patient might continue to experience devastating symptoms and likely be at risk for suicide. Though the promise of precision medicine is increasingly discussed [39], the search for clinical predictors of response to lithium treatment actually dates back to the first studies of lithium's prophylactic effect in mood disorders [40]. Indeed, Kleindienst and colleagues [17, 18] carried out two comprehensive systematic reviews of predictors of lithium response in 2005 in which they identified nearly 2,000 studies published between 1966 and 2003. They identified several demographic, psychosocial and clinical predictors that appeared to be associated with response, but the results were not always consistent and the observed effect sizes tended to be relatively small. Later, Tighe and colleagues re-

examined the data from Kleindeinst and colleagues along with new data that has since been published and generally re-affirmed the conclusions [19]. Few of the previous studies used a prospective designed to investigate predictors of lithium treatment. In Chapter 4, I analyze data from the Pharmacogenetics of Bipolar Disorder (PGBD), one of the first prospective trials of lithium monotherapy explicitly designed to identify clinical and genetic predictors of lithium response.

2.4 Predictors of genetic response to lithium treatment

Accumulating evidence suggests that genetics play an important role in differential response to lithium treatment among patients with BD. Genetic characterization can potentially aid the stratification of patients with BD, prior to initiation of treatment, into those who respond to lithium and those who do not [41]. To date, five GWAS of lithium treatment have been carried out [8, 20-22, 42]. The results have been inconsistent, and the variants identified confer relatively small increments in risk. One study tested for associations with disease based polygenic risk scores (PRS) [41], which can aggregate the contributions of many variants to response, and suggests the potential for more translational research involving PRS aimed at personalized prescribing of lithium. In Chapter 5, I use genome-wide SNP genotype data from the PGBD to test lead SNPs and PRS as genetic predictors of lithium response in a study that was designed to follow patients on lithium monotherapy to better isolate treatment effects specific to lithium. In addition, I carry out a transcription-wide association study (or TWAS), which seeks to identify associations with genes by testing variability in genetically determined gene expression [43]. Despite recently stated vulnerabilities [44], the TWAS approach aims to identify associated

features underlying lithium response directly, rather than single nucleotide variants with often ambiguous or uncertain annotation and functionality. This TWAS approach may also increase the power over traditional GWAS to identify genetic associations by reducing the burden of multiple testing. The proposed study uses one of the largest available samples of post-mortem brains from the Lieber Institute collection to generate more powerful gene expression prediction models specifically in brain regions that have been implicated in BD.

2.5 Public health relevance

The promise of precision medicine is that in the future we will be able to predict which treatments or treatment combinations are best suited for specific patients with BD so that treatments can be tailored to their individual needs, thus minimizing the process of trial and error to find the right medications [45]. This could have tremendous benefit in reducing the unnecessary suffering endured by individual patients and minimizing the overall burden and costs to public health. This project aims to characterize the changing trends in lithium utilization and to identify the clinical and genetic predictors of lithium response that ultimately can be used to bring the promise of precision medicine to a reality for patients with BD.

Chapter 3

Trends in Reports of Lithium and Other Medications for Patients with Bipolar Disorder in Office-based Practices in the United States: 1996-2015

3.1 Abstract

Background

Studies have shown that rates of lithium use for bipolar disorder in the United States declined through the 1990s as other mood stabilizing anticonvulsants and second-generation antipsychotics (SGAs) became more popular. We examined recent prescribing trends of medications for bipolar disorder from 1996 to 2015.

Methods

Twenty years of data from the National Ambulatory Medical Care Survey (NAMCS) were used. Weighted percentages of reported use of lithium, anticonvulsants, SGAs and antidepressants were calculated over two-year intervals. Logistic regression was used to examine factors related to polytherapy.

Results

Reported use of lithium declined from 38.1% (95%CI: 29.8% - 46.3%) in 1996-97 to 14.3% (95%CI: 10.6% - 18.1%) in 2006-07 and has remained stable since. During this time, reports of SGAs more than doubled. SGAs and/or anticonvulsants were reported in 75.4% (95%CI: 69.5% - 81.3%) of visits with bipolar diagnoses in 2014-15. Polytherapy increased by approximately 3% every two years and in 2014-15 occurred in over 30% of visits. Antidepressants were reported in 40-50% of visits, but their reported use without other mood stabilizers decreased from 18.2% (95%CI: 11.7% - 24.8%) in 1998-99 to 7.5% (95%CI: 4.2% - 10.9%) in 2014-15.

Limitations

The sample had limited power to study the effect of individual medications or the potential for differing effects in certain subgroups of patients.

Conclusions

This study further documents the declining use of lithium for bipolar disorder, and corresponding increase in use of anticonvulsants and SGAs, despite the fact that lithium is typically recommended as a first line therapy for bipolar disorder.

3.2 Introduction

Bipolar disorder is a severe and often disabling psychiatric disorder that affects approximately 1-4.4% of the world's population (Merikangas et al., 2007; Merikangas et al., 2011). Bipolar disorder is classically described as having a course of illness that alternates between extreme mood states of mania and depression and is characterized by episodic relapses along with longstanding disability (Nierenberg et al., 2013). Bipolar disorder is a major cause of hospitalizations and health care expenditures, and is also associated with an increased risk of suicide (Chesney, Goodwin, & Fazel, 2014). Although a number of medications have been approved for treatment of acute episodes or for maintenance treatment of bipolar disorder, lithium, the first medication shown to be a mood stabilizer in 1949 (Cade, 1949), remains a first line therapy for bipolar disorder (Goodwin et al., 2016). However, it is acknowledged that the efficacy of lithium varies widely, with about 30% of treated patients fully responding (Alda, 2017), while another 30% show only partial response and 25% no response (Hou et al., 2016). In addition, lithium use is associated with certain serious side effects (McKnight et al., 2012), requiring careful clinical management due to its narrow therapeutic index (Okusa & Crystal, 1994).

These factors, along with aggressive marketing by the pharmaceutical industry (Nassir Ghaemi, Shirzadi, & Filkowski, 2008), have led to the increasing use of other medications that also have demonstrated efficacy in bipolar disorder, including certain anticonvulsants, second-generation antipsychotics (SGAs), and antidepressants (Geddes, Calabrese, & Goodwin, 2009; Gitlin, 2018; Lindstrom, Lindstrom, Nilsson, & Hoistad, 2017; Weisler, Cutler, Ballenger, Post, & Ketter, 2006). However, anticonvulsants and antipsychotics may be less effective than lithium in preventing the recurrence of mood episodes or in reducing the overall risk of suicide (Baldessarini & Tondo, 2009; Caley,

Perriello, & Golden, 2018; Kessing, Sondergard, Kvist, & Andersen, 2005). In addition, the appropriate clinical use of antidepressants in bipolar disorder remains uncertain (Gitlin, 2018; Sachs et al., 2007).

There has also been an increase in polytherapy with combinations of the different medications. There is some evidence that certain combinations of medications may be superior to monotherapy for treating manic episodes, but continued polytherapy after the manic phases resolve remains controversial (Geoffroy, Etain, Henry, & Bellivier, 2012). In addition, there is inconsistent evidence for treating the depressive episodes with different medication combinations in comparison to monotherapy (Lin, Mok, & Yatham, 2006). Reports of increased side effects (Brooks et al., 2011) and concerns of potential drug-drug interactions (Dunner, 2003) with polytherapy further raise questions about treatment choices.

The changing trends over time in the use of various medications for treatment of bipolar disorder have been documented by several studies in European countries, the results from which have suggested a constant or even an increasing trend of lithium use (Bramness, Weitoft, & Hallas, 2009; Castells et al., 2006; Hayes et al., 2011; Wilting, Souverein, Nolen, Egberts, & Heerdink, 2008). In the US, time trends in prescriptions of lithium and other medications for treatment of bipolar disorder have been examined through the 1990s and early 2000s using data from the National Ambulatory Medical Care Survey (NAMCS) and Kaiser Permanente, Northern California health system (Blanco, Laje, Olfson, Marcus, & Pincus, 2002; Hunkeler et al., 2005; Moreno et al., 2007). However, there is little national level data on time trends over the past 20 years. Data from a single tertiary care clinic

suggested a decrease in lithium usage (Hooshmand et al., 2014); however, it is unclear whether this trend is generalizable to other settings.

In this study, we examined time trends in prescriptions of lithium and other medications for bipolar disorder in a representative sample across the US over a period of 20 years. We used data from NAMCS, a well-characterized national survey of US office-based practices of physicians from different specialties, conducted annually across a broad range of office settings. Specifically, we examined time trends in prescriptions of lithium, mood-stabilizing anticonvulsants, SGAs, and antidepressants in visits with a diagnosis of bipolar disorder. The results of our study provide useful data to characterize the changing patterns in the management of bipolar disorder in the US over the past two decades.

3.3 Methods

Study Data

For the current analysis, we used 20 years of data from the NAMCS between 1996 to 2015 to summarize and analyze recent time trends in prescriptions of lithium and other medications for bipolar disorder. The NAMCS has been conducted annually since 1989 utilizing a multistage probability design in which a sample of patient visits are randomly selected with a pre-specified probability from within certain physician practices, which in turn are randomly selected with a pre-specified probability from within primary sampling units (PSUs) across the country (CDC, 2015a). Data are obtained on patient, visit, and physician practice characteristics (CDC, 2017). The current analysis was considered

exempt from human subject research, because the data were publicly available and de-identified.

We identified all visits in which the patient had a diagnosis of bipolar disorder as indicated by a primary, secondary, or tertiary diagnosis with one of the following ICD-9 codes: 296.00-296.06, 296.10-296.16, 296.40-296.46, 296.50-296.56, 296.60-296.66, 296.7, 296.80-296.81, and 296.89. While the number of diagnoses that could be recorded per visit increased over the years, we extracted the first three diagnoses for each encounter to be consistent across all years.

All prescription and non-prescription medications were recorded by physicians for each patient visit. Physicians recorded all new medications as well as those patients were specifically instructed or expected to continue. All medications were coded using the National Drug Code Directory (NDCD) prior to 2006 and the Multum drug dictionary starting in 2006 (CDC, 2015b). While the number of medications that could be recorded per visit in NAMCS has increased over the years, we extracted the first six medications for each encounter to be consistent across all years.

We categorized medications taken for bipolar disorder based on their NDCD or Multum codes into the following classes: lithium, anticonvulsants used as mood stabilizers (valproic acid [divalproex sodium], carbamazepine, and lamotrigine), second-generation antipsychotics (SGAs) (aripiprazole, asenapine maleate, clozapine, iloperidone, lurasidone, olanzapine, olanzapine/fluoxetine, paliperidone, quetiapine, risperidone, and ziprasidone), and antidepressants (amitriptyline, amoxapine, chlordiazepoxide/amitriptyline, citalopram, clomipramine, desipramine HCl, duloxetine, escitalopram oxalate, fluvoxamine maleate, hydrobromide, isocarboxazid, maprotiline, nefazodone HCl, nortriptyline,

olanzapine/fluoxetine, paroxetine HCl, paroxetine mesylate, perphenazine/amitriptyline, phenelzine sulfate, selegiline, tranylcypromine sulfate, trazodone HCl, and venlafaxine HCl). We considered patients as receiving “no medication treatment” for bipolar disorder at a visit if they were not reported taking any of the above medications at that visit.

Patient characteristics included sex (male versus female), self-reported race (Caucasian, African-American, or others), and age (adults versus children, with patients under the age of 18 considered as children). The visit type was defined as either “psychiatry” if the visit was to a psychiatrist, versus “others” for all other office-based physicians. The insurance type used to cover visits was classified as either private insurance, Medicaid/Children’s Health Insurance Program (CHIP), Medicare, self-pay, or others.

Data Analysis

Visits in which lithium was reported were compared to those in which other or no mood stabilizer medications were reported with regard to the patient characteristics, the visit type, the insurance type, and antidepressants using contingency tables and chi-square tests. Time trends in reports of the different medication classes for bipolar disorder were described as weighted percentages calculated from the number of visits in which a particular category of medication was reported out of the total number of bipolar disorder visits in a given year. We further examined time trends in reports of the different mood stabilizer classes within important patient sub-groups, including by bipolar disorder subtypes and age, as well as time trends in reports of the top three most used anticonvulsants and SGAs. Next, we used logistic regression to examine the association between patient and visit characteristics and polytherapy of mood stabilizer medications

(i.e., lithium, anticonvulsants and/or SGAs). In the logistic regression analyses, we included only bipolar disorder visits in which medications from any of the mood stabilizer classes were reported. All analyses were adjusted for visit weights, clustering, and stratification of data using design elements provided by the National Center for Health Statistics that allow generalization of the NAMCS data to the US population. In order to obtain more stable estimates of the frequency of drug reports, we combined visit data over two-year intervals. All analyses were conducted in RStudio (Version 1.1.447).

3.4 Results

Sample Characteristics

Between 1996 and 2015, the NAMCS included data on 645,784 visits to office-based physicians; of these, 5,400 (0.6%) visits involved patients who had a bipolar disorder diagnosis, including approximately 47% with bipolar I disorder, 13% with bipolar II disorder, and 40% with bipolar disorder not otherwise specified. Table 1 summarizes the characteristics of these bipolar disorder visits and compares them by whether lithium, other mood stabilizers (anticonvulsants and/or SGAs), or no mood stabilizers were reported. The majority of visits involved Caucasian adults, and just over 60% were with females. Over three-quarters of the visits were with psychiatrists, and over 40% were covered by private insurance, while another 35% were covered by Medicare and/or Medicaid. Lithium was reported in approximately 17% of bipolar disorder visits, other mood stabilizers (anticonvulsants and/or SGAs) were reported in 52%, and no mood stabilizers were reported in 31%. There were significant differences in race, age, visit type, insurance type, and reports of antidepressants across the 3 treatment groups (all p-values < 0.0001).

Reports of any mood stabilizers were more likely to occur at psychiatry visits. Reports of lithium were more likely to involve Caucasian adult patients who were self-paying and not on Medicaid. Compared to lithium, reports of other mood stabilizers were more likely among children and to be accompanied with reports of antidepressants.

Time Trends in Mood Stabilizer Classes

The percentage of bipolar disorder visits among all visits steadily increased over the study period from 0.3% (95% CI: 0.2% - 0.4%) in 1996-97 to 0.8% (95% CI: 0.6% - 1.0%) in 2014-15. Among the bipolar disorder visits, the percentage in which any mood stabilizer was reported remained stable at approximately 70%, until 2014-15, when there was an increase to 79.4% (95% CI: 73.9% - 85.0%). However, during this period there was a consistent decline in reports of lithium (Figure 1). The percentage decreased from 38.1% (95% CI: 29.8% - 46.3%) in 1996-97 to 14.3% (95% CI: 10.6% - 18.1%) in 2006-07, and it remained steady at approximately 14% from 2008-09 on. During this period, there was a parallel increase in reports of SGAs, which more than doubled from 19.2% (95% CI: 12.4% - 26.0%) in 1996-97 to 48.0% (95% CI: 42.2% - 53.9%) in 2008-09 and reached a peak of 52.2% (95% CI: 43.9% - 60.5%) in 2014-15. Reports of anticonvulsants for bipolar disorder remained generally stable at around 40%, with a low of 31.0% (95% CI: 23.9% - 38.1%) in 1996-97 and a recent peak of 43.3% (95% CI: 36.9% - 49.7%) in 2014-15. Overall, by 2014-15, other mood stabilizers (anticonvulsants and/or SGAs) were reported in 75.4% (95% CI: 69.5% - 81.3%) of bipolar disorder visits.

Time Trends by Cohort Sub-Groups

Trends over time in reports of different mood stabilizer classes were generally similar for patients with different bipolar disorder subtypes, except for a somewhat greater overall reports of lithium for bipolar I disorder compared to other bipolar diagnoses (Supplementary Figure 1). By the end of 2014-15, lithium was reported for 19.5% (95% CI: 11.0% - 27.9%) of visits for patients with bipolar I disorder compared to only 11.6% (95% CI: 7.0% - 16.2%) of visits for patients with other bipolar diagnoses.

Trends over time in reports of the different classes of mood stabilizers were also generally similar when comparing adults with children (age < 18 years old), except for two noteworthy differences. First there was greater variability in estimates over time for children due to the smaller sample size, and second, there was a notable difference in SGAs reports (Supplementary Figure 2). The decline in reports of SGAs seen after 2008-09 occurred in both adults and children, but was more precipitous in children, where reports of SGAs dropped from a high of 70.9% (95% CI: 55.0% - 86.7%) in 2008-09 to 43.2% (95% CI: 26.1% - 60.3%) in 2012-13. However, reports of SGAs in children rebounded in 2014-15 to a high of 78.4% (95% CI: 50.2% - 100%), although given the wide confidence intervals, it is unclear if this reflects a genuine change or is due to unstable estimates from a small sample size.

Time Trends in Specific Anticonvulsants and SGAs

Trends over time in reports of the top three most prescribed anticonvulsants and SGAs between 1996-2015 are shown in Figure 2. Among anticonvulsants, there was a notable decline in reports of valproic acid/divalproex sodium coupled with an increase in lamotrigine. The percentage of bipolar disorder visits with reports of valproic

acid/divalproex sodium peaked in 1998-99 at almost 35%, but then declined to around 14% in 2014-15. Conversely, as of 2006-07 lamotrigine became the most widely reported anticonvulsant and was reported in approximately 28% of bipolar disorder visits in 2014-15. Among SGAs, there was a steady increase in the percentage of bipolar disorder visits with reports of quetiapine and aripiprazole since their introduction. Beginning in 2004-05, quetiapine became the most widely reported SGA, being reported in 22.0% (95% CI: 15.3% - 28.7%) of bipolar disorder visits in 2014-15. Since 2008-09, aripiprazole has been the second most widely reported SGA and was reported in 12.3% (95% CI: 7.4% - 17.2%) of bipolar disorder visits in 2014-15.

Time Trends in Polytherapy

The use of multiple medications to treat mood in bipolar disorder has steadily increased since 1996-97. In 1996-97, two or more mood stabilizers were reported in only 20.0% (95% CI: 13.1% - 26.8%) of bipolar disorder visits; however, by 2014-15 the percentage had gone up to 33.6% (95% CI: 24.8% - 42.4%) (Figure 3). Among bipolar disorder visits in which any mood stabilizer treatment was reported, the odds of polytherapy with mood stabilizers increased by 1.03 times (95% CI: 0.98 - 1.08) every two years, after controlling for sex, race, age, visit type, and insurance type. Of these other covariates, the provider type was significantly associated with increased polytherapy, the odds being 3.16 (95% CI: 2.12 - 4.71) times greater in psychiatric versus non-psychiatric visits.

Despite the general increase in polytherapy of mood stabilizers over the past two decades, reports of lithium concomitantly with other mood stabilizers declined somewhat

from a high of 13.8% (95% CI: 8.6% - 19.0%) in 1996-97 to 10.7% (95% CI: 6.5% - 15.0%) in 2014-15. Over this same time, reports of lithium monotherapy decreased even more dramatically from 24.3% (95% CI: 18.0% - 30.6%) in 1996-97 to only 4.0% of bipolar disorder visits (95% CI: 2.3% - 5.7%) in 2014-15.

Time Trends in Antidepressants

Antidepressants were consistently reported in approximately 40-50% of bipolar disorder visits (Figure 4). Moreover, the percentage of visits in which antidepressants were reported by themselves without any mood stabilizers decreased from 18.2% (95% CI: 11.7% - 24.8%) in 1998-99 to 7.5% (95% CI: 4.2% - 10.9%) in 2014-15. At the same time, the percentage of visits in which antidepressants were reported with mood stabilizer(s) steadily increased from 26.3% (95% CI: 20.2% - 32.3%) in 1996-97 to 45.3% (95% CI: 38.1% - 52.6%) in 2014-15. Reports of antidepressants were generally lower in children (Supplementary Figure 2), ranging from 20-40% for most of the past two decades, compared to 40-50% in adults. However, in 2014-15, the percentage notably increased in children to the same level as adults, reaching above 50% for both.

3.5 Discussion

Lithium, the first medication identified to be effective for treating bipolar disorder, remains a first line therapy (Severus, Schaaff, & Moller, 2012). However, the use of lithium has been steadily declining over the past thirty years. The results reported in this paper further document the decline in reports of lithium use to approximately 15% of physician office visits for bipolar disorder in the US. In its place, there has been a notable increase in

reported use of mood stabilizing anticonvulsants, initially valproic acid, followed by lamotrigine, and more recently the SGAs, particularly quetiapine and aripiprazole. Mood stabilizing anticonvulsants and SGAs are each now reported in approximately 40-50% of bipolar disorder visits, and they are concomitantly reported in over 20% of these visits. Moreover, we found that polytherapy with mood stabilizing treatments for bipolar disorder has steadily increased since 1998-99, such that two or more of any mood stabilizer medications are now reported in over 30% of bipolar disorder visits. By contrast, reports of lithium monotherapy have steadily declined over this time period to approximately 4% of bipolar disorder visits.

The current findings are consistent with those from previous studies in the US. An earlier analysis of the NAMCS data using only outpatient psychiatrist visits found that prescriptions of lithium declined from 50.9% (95%CI: 47.0% - 54.8%) in 1992-95 to 30.1% (95%CI: 26.5% - 33.7%) in 1996-99 (Blanco et al., 2002). Our findings extend this observation into the first two decades of the 2000's. In addition, a smaller study of patients referred to a tertiary care referral clinic found that lamotrigine, quetiapine, and aripiprazole use more than doubled from 2000-05 to 2006-11, while lithium, valproate, olanzapine and risperidone use decreased (Hooshmand et al., 2014). Our results show similar time trends at the national level.

Studies of time trends in lithium use have been conducted in several different international countries as well. These include studies from Spain using annual lithium purchase data between 1985-2003 from the ECOM database of the Spanish Ministry of Health (Castells et al., 2006); the Netherlands using outpatient data between 1996-2005 obtained from the PHARmacoMORbidity (PHARMO) record linkage system (Wilting,

Souverein, Nolen, Egberts, & Heerdink, 2008); Scandinavia using prescription data between 2005-06 from three Sweden, Norway and Denmark prescription databases (Bramness, Weitoft, & Hallas, 2009); and the United Kingdom using primary care data between 1995-2009 from the Health Improvement Network (THIN) (Hayes et al., 2011). Interestingly, studies from these other countries have suggested a constant, or even increasing, rate of lithium use. For example, the UK study reported the proportion time in treatment with lithium remained constant around 30% across 1995-2009 (Hayes et al., 2011). Many of these studies, however, covered either an earlier (all before 2009) or a shorter time frame than the current study, which might partly explain the differences observed with our findings.

Our study used data from a nationally representative sample of office based clinical visits across the US. In addition, these data covered a twenty-year period from 1996-2015 and were collected using largely consistent procedures. This allowed us to have a relatively large sample to make statistical inferences, which is an advantage over past bipolar disorder studies. By combining the data into two-year intervals, we attempted to obtain more stable results while not losing the ability to observe time trends. Nevertheless, the study sample may not have been sufficiently large to detect time trends in subgroups of patients or time trends specific to individual medications. In addition, the study relied on physician assigned diagnoses that were not validated, which raises the possibility of diagnosis errors. This might be especially problematic because of the high rate of mis-diagnoses for bipolar disorder (Ruggero, Zimmerman, Chelminski, & Young, 2010). Indeed, we observed an unusually high rate of bipolar disorder not otherwise specified, which may reflect some of the ambiguities in the diagnosis. To the extent there is error in the diagnosis of bipolar

disorder and its sub-types, the rates of reported medication use may be mis-leading. Furthermore, the dataset does not distinguish patients with bipolar disorder that may be in different mood states. As a result, we were unable to determine whether the different medications were being taken for mania, depression, or maintenance. Finally, we did not have any information on adherence, so we can only comment on what medications patients were supposed to be taking.

Despite these limitations, the declining use of lithium that we observed in the US is striking. It is also interesting to note that the use of lithium is reported less frequently among African Americans and for patients on Medicaid, which may reflect disparities in access to health care (Akinhanmi et al., 2018). Lithium has been shown to be an effective treatment for bipolar disorder, and it is typically recommended among first-line treatments for bipolar disorder, including in the manic, depressive and maintenance phases (Yatham et al., 2018). Despite this, there was a notable increase in the reported use of alternative mood stabilizers, including anticonvulsants and SGAs, at the apparent expense of lithium. However, there is limited evidence regarding the comparative safety and effectiveness of these medications. The increasing polytherapy with mood stabilizers that we observed has also been documented in bipolar disorder samples from other countries and in psychiatry visits overall (Hayes et al., 2011; Mojtabai & Olfson, 2010). This is of interest because there may be even less empirical evidence to guide which combinations of mood stabilizers are most effective despite increased risk for side effects (Brooks et al., 2011). On the encouraging side, we did observe a decline in the reported use of antidepressants without mood stabilizers, which is consistent with guidelines to avoid antidepressant monotherapy to treat bipolar depression (Yatham et al., 2018). Overall, given the limited evidence to

help guide which of the treatment options will work best for different patients with bipolar disorder, further research is needed to achieve the promise of precision medicine to better predict which medications or medication combinations are most effective for individual patients, thus minimizing the typical process of trial and error of finding the right medications.

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Table 1. Characteristics of visits with bipolar disorder diagnoses by mood stabilizer treatment ⁱ

	Bipolar (n=5,400)	Lithium ⁱⁱ (n=925)	Other Mood Stabilizers ⁱⁱⁱ (n=2,782)	No Mood Stabilizers (n=1,693)	p-value ^{iv}
Sex, (%)					0.15
Female	61.6	58.8	62.0	62.5	
Male	38.4	41.2	38.0	37.5	
Race, (%)					<0.0001 ^{v, vi}
Caucasian	88.1	91.5	86.9	88.9	
African-American	7.9	3.7	8.8	8.2	
Others	4.0	4.8	4.3	2.9	
Age, (%)					<0.0001 ^{v, vii}
Adults (>=18 years)	91.6	94.4	89.3	93.8	
Children	8.4	5.6	10.7	6.2	
Visit Type, (%)					<0.0001 ^{vi, vii}
Psychiatry	76.8	83.0	83.5	62.3	
Others	23.2	17.0	16.5	37.7	
Insurance Type, (%)					<0.0001 ^{v, vi}
Private Insurance	43.9	43.9	44.7	42.7	
Medicaid, CHIP	17.3	11.8	19.0	17.5	
Medicare	17.6	19.3	16.2	19.0	
Self-pay	13.6	16.6	13.0	13.1	
Others	7.6	8.3	7.2	7.8	
Antidepressants, (%)					<0.0001 ^{v, vii}
Yes	46.9	42.3	54.1	38.4	
No	53.1	57.7	45.9	61.6	

ⁱ All numbers shown in the table are weighted percentages (see **Methods**).

ⁱⁱ Among visits being prescribe lithium, 450 were prescribed lithium monotherapy (as the only mood stabilizer), 129 were concomitantly prescribed anticonvulsants (but no SGAs), 243 SGAs (but no anticonvulsants), and 103 both.

ⁱⁱⁱ Among visits being prescribed non-lithium mood stabilizers, 1,003 were prescribed anticonvulsants as the only mood stabilizers, and 1,094 SGAs. 685 were concomitantly prescribed anticonvulsants and SGAs.

^{iv} P-values are from Chi-squared tests comparing percentages across the three treatment groups.

^v Chi-squared tests p<0.017 (0.05/3) comparing Lithium vs Other Mood Stabilizers

^{vi} Chi-squared tests p<0.017 (0.05/3) comparing Lithium vs No Mood Stabilizers

^{vii} Chi-squared tests p<0.017 (0.05/3) comparing Other Mood Stabilizers vs No Mood Stabilizers

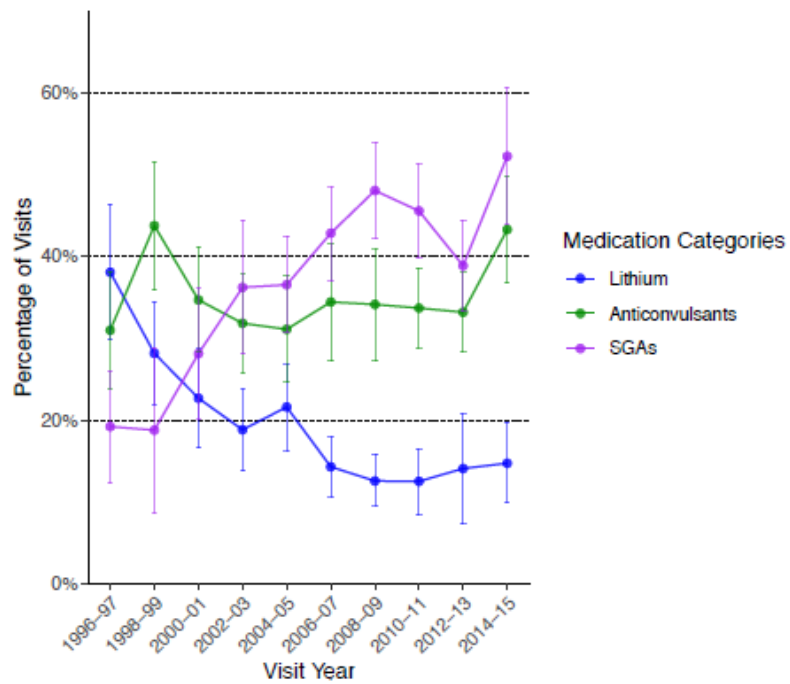


Figure 1. Trends between 1996-2015 in the percentage of visits in which different categories of mood stabilizer medications were reported bipolar disorder. Shown are percentages among visits with a diagnosis of bipolar disorder. Error bars show 95% confidence intervals for each weighted estimate of the percentage of visits.

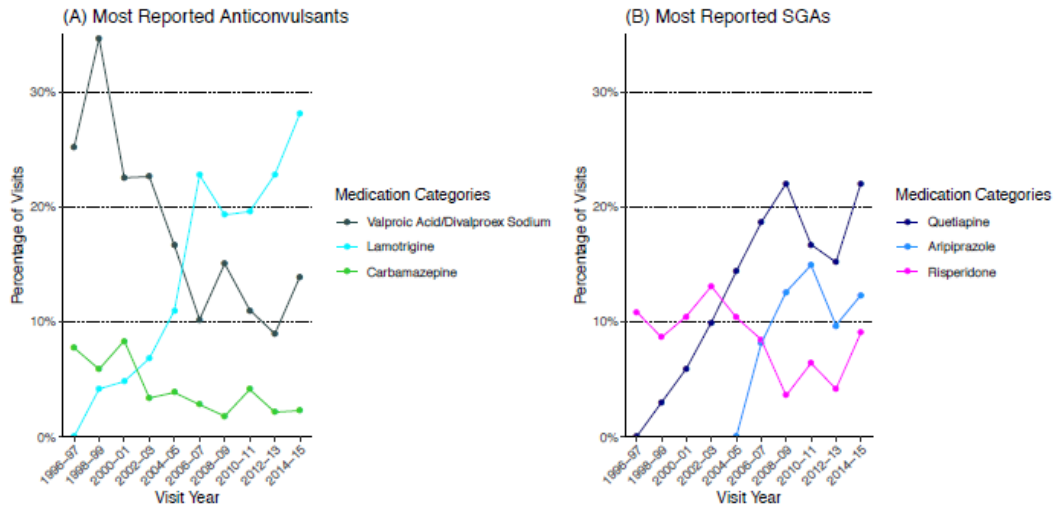


Figure 2. Trends between 1996-2015 in the percentage of visits in which specific (A) anticonvulsants and (B) SGAs were reported. Aripiprazole entered the US market later than 1996, which explains why aripiprazole first appears in the NAMCS data in 2004-05. Shown are percentages among visits with a diagnosis of bipolar disorder. Error bars are not shown due to smaller sample sizes of the stratified results.

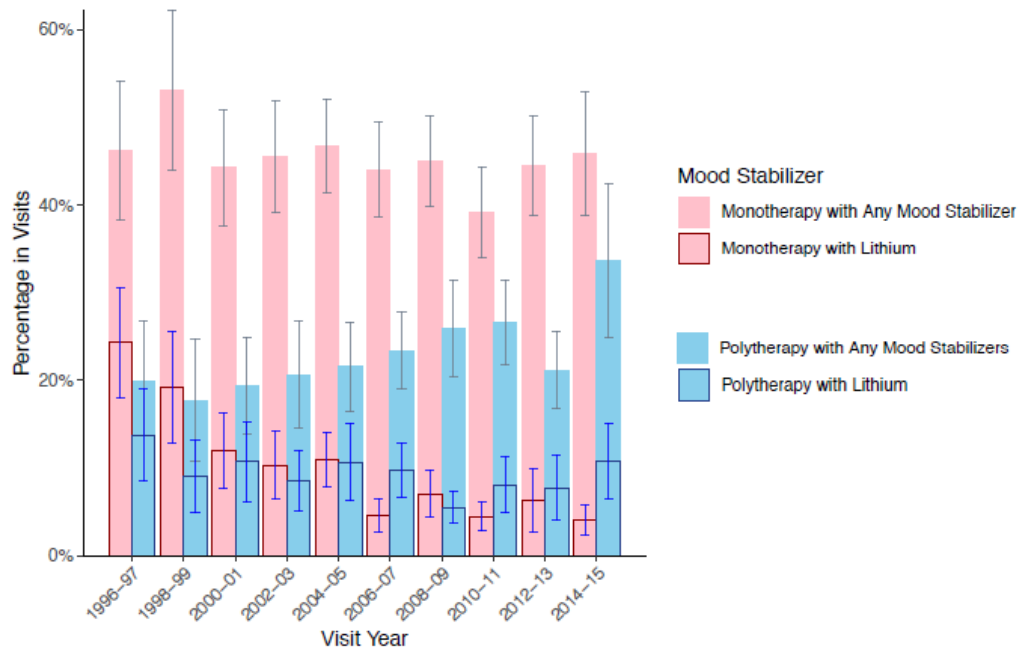


Figure 3. Trends between 1996-2015 in percentage of visits in which monotherapy vs polytherapy of mood stabilizer medications was reported. Mood stabilizers refer to lithium, anticonvulsants, or second-generation antipsychotics; monotherapy means taking only one of these medications for bipolar disorder, while polytherapy means taking any combination of more than one of these medications. Shown are percentages among visits with a diagnosis of bipolar disorder. Error bars show 95% confidence intervals for each weighted estimate of the percentage of visits.

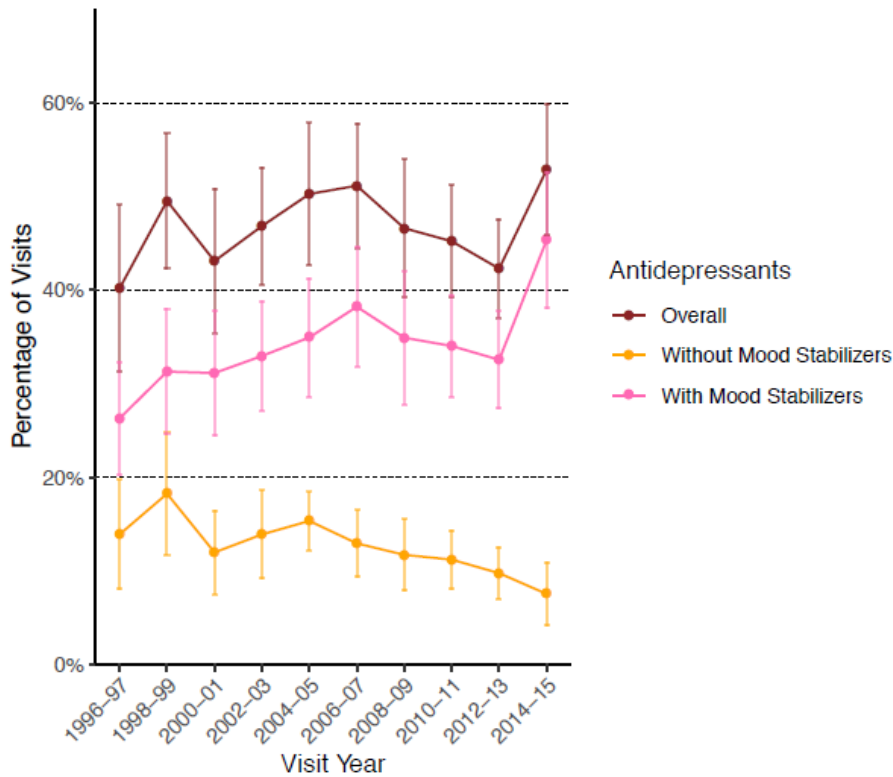
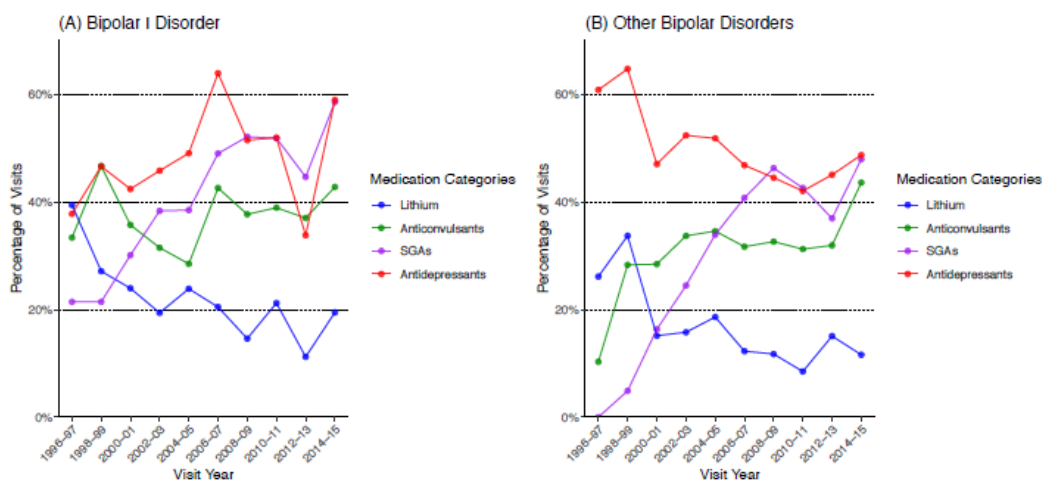
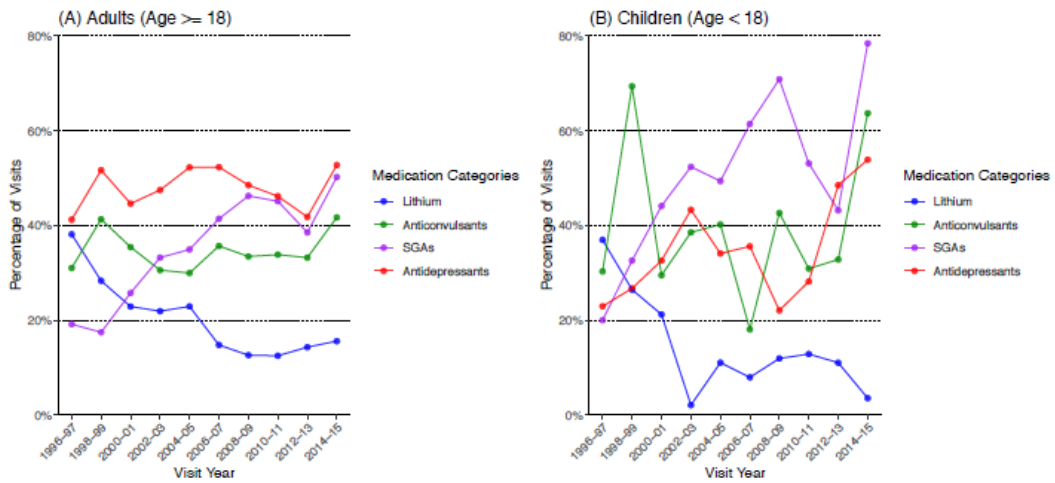


Figure 4. Trends between 1996-2015 in percentage of visits in which antidepressants were reported. Shown are percentages among visits with a diagnosis of bipolar disorder. Error bars show 95% confidence intervals for each weighted estimate of the percentage of visits.



Supplemental Figure 1. Trends between 1996-2015 in percentage of visits in which different categories of medications were reported by bipolar disorder sub-type, (A) bipolar I disorder vs (B) other bipolar disorders. Shown are percentages among visits with a diagnosis of bipolar disorder. Error bars are not shown due to smaller sample sizes of the stratified results.



Supplemental Figure 2. Trends between 1996-2015 in percentage of visits in which different categories of medications were reported for (A) adults vs (B) children. Shown are percentages among visits with a diagnosis of bipolar disorder. Error bars are not shown due to smaller sample sizes of the stratified results.

Chapter 4

Clinical Predictors of Response to Lithium Treatment in The Pharmacogenomics of Bipolar Disorder (PGBD) Study

4.1 Abstract

Background

Lithium is regarded as a first line treatment for bipolar disorder (BD), but partial response and non-response commonly occurs. There exists a need to identify lithium non-responders prior to initiating treatment. The Pharmacogenomics of Bipolar Disorder (PGBD) Study was designed to identify clinical and genetic predictors of lithium response.

Methods

The PGBD Study was an eleven-site prospective trial of lithium treatment in bipolar 1 disorder. Subjects were stabilized on lithium monotherapy over four months and gradually discontinued from all other psychotropic medications. After ensuring a sustained clinical remission (defined by a score of <3 on the CGI for four weeks) had been achieved, subjects were followed for up to two years to monitor clinical response. Cox proportional hazard models were used to examine the relationship between clinical measures and time until failure to remit or relapse.

Results

A total of 345 individuals were enrolled into the study and included in the analysis. Of these, 101 subjects failed to remit or relapsed, 88 achieved remission and continued to study completion, and 156 were terminated from the study for other reasons. Significant clinical predictors of treatment failure ($p < 0.05$) included baseline anxiety symptoms, functional impairments, negative life events and lifetime clinical features such as a history of migraine, suicidal ideation/attempts, and mixed episodes, as well as a chronic course of illness.

Conclusions

In this PGBD Study of lithium response, several clinical features were found to be associated with failure to respond to lithium. Future validation is needed to confirm these clinical and genetic predictors of treatment failure and their use clinically to distinguish who will do well on lithium before starting therapy.

4.2 Introduction

Lithium is regarded as a first-line treatment for bipolar disorder (BD) (1-3), but it does not work for all patients. The modern use of lithium for treatment of BD was first introduced by John Cade in 1949, and it has been widely studied since. Although findings from these studies have been controversial, the evidence for the efficacy of lithium in acute mania and maintenance treatment is well established. In a meta-analysis of five randomized controlled trials of BD comparing prophylactic lithium therapy with placebo, Geddes and colleagues (4) found that lithium is more effective than placebo in preventing recurrence of illness, with 60% in the lithium group remaining well over 1–2 years compared with 40% in the placebo group. In a subsequent meta-analysis of six studies of lithium in the treatment of acute mania, Yildiz and colleagues found that 48% of patients responded to lithium compared to 31% for placebo (5). While these seminal reviews unequivocally demonstrate the efficacy of lithium for both acute mania and maintenance treatment of BD, they also highlight that anywhere from 40-50% of patients do not respond adequately over a two year period and require either the addition of or a change to another psychotropic drug (5). These findings are consistent with observational data from longitudinal cohort studies (6-8).

There is considerable continued interest in identifying predictors of response to lithium before starting treatment in order to avoid the typical trial and error process of finding the right medication for a particular patient during which time he or she may continue to experience devastating symptoms and be at risk for suicide. This is the goal of precision medicine. Although the promise of precision medicine has garnered a great deal of attention recently (9), the search for predictors of lithium response dates back to the very first studies of its prophylactic effect in mood disorders (10). Previous studies have focused

on socio-demographic or clinical correlates of response, but there is increasing effort to identify relevant biological markers, including biomarkers from neuroimaging, neurophysiology or molecular studies. The evidence from these studies to date is largely conflicting.

The Pharmacogenomics of Bipolar Disorder (PGBD) Study (www.clinicaltrials.gov, NCT01272531) is one of the first prospective studies of lithium treatment designed to prospectively identify clinical and molecular predictors of lithium response. We report here the results of an analysis of data from this study to examine clinical predictors.

4.3 Methods

Study Overview

The PGBD was one of fourteen research projects in the Pharmacogenetic Research Network funded by the National Institute of Health to support multi-disciplinary, collaborative research on how genetic factors contribute to inter-individual differences in responses to medications. The PGBD set out to conduct a multi-site prospective study of lithium monotherapy in the treatment of BD.

The details of the trial have been described elsewhere (11). Briefly, the goal of the study was prevention of illness recurrence by lithium monotherapy. All patients were observed in an observation phase lasting 4 weeks to confirm they were in remission defined by having a Clinical Global Impression of Severity Scale (CGI-S) score of <3 (mildly ill) for at least 4 weeks. After the observation phase, the patients entered a two-year maintenance phase, during which they were assessed every 2 months to monitor their ongoing clinical response. Patients who came into the trial clinically unstable and/or not on

lithium monotherapy were first transitioned to lithium monotherapy in a stabilization phase that lasted a maximum of 16 weeks which included visits every other week for the first 8 weeks and one visit per month for the next two months. Throughout the follow-up, patients were allowed to take a benzodiazepine for anxiety and/or zolpidem for sleep. For investigation of genetic and other molecular predictors of response to lithium treatment, blood was collected from all patients and a skin biopsy on a subset of patients. A range of clinical measures (described below) was collected at the screening and subsequent visits to monitor clinical progress and enable investigation of clinical predictors of response.

Participants

Patients were enrolled into the study at nine sites within the United States and two international sites. The nine domestic sites included: University of California, San Diego; Indiana University; University of Chicago; University of Pennsylvania; University of Iowa; Johns Hopkins University; Case Western Reserve University; University of Michigan; and the Mayo Clinic. The two international sites were University of Bergen, Norway, and Dalhousie University in Halifax, Canada.

Patients were included in the study if they: 1) had bipolar I disorder in any phase of illness; 2) were naïve to or not presently on lithium and had at least one affective episode meeting DSM-IV criteria in the last 12 months or were currently on lithium and did not have any history of mood episodes meeting DSM-IV criteria in the last 6 months; 3) were able to give informed consent; 4) were 18 years or older; and 5) were currently symptomatic, as defined as a CGI-S score of at least 3 (mild severity), unless the patient entered the study already stable on lithium monotherapy. Women of child bearing potential

were included if they agreed to use adequate contraception and inform their doctor at the earliest possible time of their plans to conceive.

Patients were excluded if they: 1) were unwilling or unable to comply with study requirements; 2) had renal impairment (serum creatinine >1.5 mg/dL); 3) had thyroid stimulating hormone (TSH) over >20% above the upper normal limit or, if on thyroid medication, had not been euthyroid for at least 3 months before the first visit; 4) were currently in crisis such that inpatient hospitalization or other crisis management should take priority; 6) met criteria for physical dependence requiring acute detoxification from alcohol, opiates or barbiturates; 7) were pregnant or breastfeeding; 8) had participated in a clinical trial of an investigational drug within the past 1 month, or 9) had a history of lithium toxicity, not due to mismanagement or overdose, that required treatment.

All study procedures were approved by local Institutional Review Boards (IRBs), and all patients provided informed consent. This analysis included data on the first 345 BD patients who enrolled into the study and completed at least four weeks of the study.

Clinical Outcomes

Patients were followed until they: 1) completed all study visits over two years of the maintenance phase (or had achieved the maintenance phase and were still active in the ongoing study by the date of the data freeze), 2) were terminated from the study before completion of all visits because of failure to achieve (i.e., failure to remit) or maintain (i.e. relapse) stabilization on lithium, or 3) were terminated from the study for other reasons.

Failure to remit was defined by the inability to achieve clinically sustained remission (where remission was documented as described above) by the end of the observation phase

or based on clinical judgment that the patient was unable to adequately stabilize on lithium monotherapy.

Relapse was evaluated using the Mood Episode Checklist which summarizes DSM-IV criteria for mania and depression and was collected at each visit during the maintenance phase. Relapse was defined by the following: 1) meets criteria for mania and has a CGI-S of 5 (markedly ill) or greater; 2) meets criteria for a major depressive episode with 4 week duration; 3) meets criteria for a mixed episode with CGI-S of 5 or greater; 4) psychiatric hospitalization for a mood episode is required; or 5) in the physician's judgment the patient cannot be managed on monotherapy and a change in medication is required. Episodes of hypomania without impairment of function were not considered relapses. These criteria were designed to be stringent so as to detect clear failures of prophylaxis, rather than brief episodes that might not require a medication change in clinical practice.

Clinical Predictors

Patients were evaluated with the Diagnostic Interview for Genetic Studies (DIGS) in order to establish a diagnosis of bipolar I disorder by DSM-IV criteria and collect detailed historical clinical information about current and lifetime mental illnesses. Patients also completed a range of self and clinician rated scales at the screening and subsequent visits to document the clinical course of illness and factors that may relate to the course. Self-rated scales included The Childhood Life Events Scale; The Lifetime History of Aggression Scale; The Columbia Suicide Symptom Severity Scale; The Basic Language Morningness Scale (BALM); the Temperament Evaluation of Memphis, Pisa, Paris and San Diego – auto-questionnaire version (TEMPS-A); the 16 item Quick Inventory of

Depression Symptomatology Self-Report (QIDS-SR-16); the Sheehan Disability Scale; the Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q); and the Life Events Questionnaire (LEQ). Clinician rated scales included the following: the Clinical Global Impressions of Severity Scale (CGI-S); the Hamilton Rating Scale for Anxiety (HAM-A); the Montgomery Asberg Depression Rating Scale (MADRS); the Clinician Administered Rating for Mania (CARS-M); and the Modified Scale for Suicidal Ideation (MSSI). From the assessments collected either at the screening or baseline visits, we derived 43 clinical variables for analysis that were selected based on clinical experience and an expert review of the literature involving three of us (JK, MA, and JC). These included variables on socio-demographic factors, baseline symptoms, clinical history and course, co-morbid illnesses, family history of mental illness, childhood and current life events, and level of functioning. See Table 1 for a full list of variables that were examined.

Statistical Analyses

Differences in socio-demographic factors between patients who completed all study procedures, those who failed to remit or experienced a relapse, and those who were terminated from the study for other reasons were compared using chi-square tests for categorical variables and one-way ANOVA for continuous variables. We then used survival analysis with Cox Proportional Hazard models to examine the relationship between clinical predictors measured at baseline and the time from study entry to treatment failure, which was defined as the time of the last visit at which the patient was determined to have failed to remit or to have relapsed. All other patients were censored at the time of their last visit in the on-going study. We examined each clinical predictor individually in

models that additionally controlled for potential confounders including age at study entry, sex, race, and lithium status upon entry into the study. These variables were selected from the available data because they are important socio-demographic factors that experience indicated may be relevant and/or they were found to differ with treatment outcome. Race was captured as a categorical variable for Caucasians, Asians, African Americans, or other. Lithium status upon entry into the study was captured as a categorical variable to distinguish those who entered the study stable on lithium monotherapy, on lithium plus other psychotropic medications, or not on lithium. We used two-tailed $p < 0.05$ to declare associations statistically significant. We did not correct for multiple testing for two reasons: the clinical predictors were carefully selected based on prior hypotheses that they may be relevant to treatment response, and we reasoned Bonferroni correction would be too conservative and we wanted to prioritize detection of true associations over rejection of false positives.

To determine if the associations with treatment response of the clinical predictors identified through the above procedures differ in the initial versus later phases of follow-up, we stratified the survival analyses and looked first at survival over the stabilization and observation phases among all patients who entered the study, and then separately over the maintenance phase among patients who entered the maintenance phase. To formally test for differences in association, we combined the stratified survival data and included in the Cox Proportional Hazard models an interaction term between the clinical predictor and an indicator variable for the stabilization/observation versus maintenance phases.

To assess the robustness of observed associations to the assumptions of the survival analysis, we carried out two additional analyses. We defined two alternative but related

response variables for analysis: 1) an acute response variable based on whether patients proceeded to the maintenance phase or not; and 2) a prophylactic response variable which contrasted patients who completed all study visits or who had reached the maintenance phase and were still active on study as of the data freeze versus those who failed to remit or who relapsed on lithium monotherapy before completing all study visits. We then used logistic regression to examine the association between the clinical predictors and the two different dichotomous response variables in models that controlled for the same potential confounders as in the survival analysis. The inferences drawn from these two alternative logistic regression analyses were nearly identical to those from the survival analysis, so we report here only the results from the survival analysis. All analyses were performed independently at two study sites to ensure the accuracy of the results.

Finally, to evaluate the predictive ability of a model that included all clinical predictors individually found to be significantly associated with treatment failure, we carried out a receiver-operating curve (ROC) analysis specifically for survival data. We first carried out multiple imputation to fill in missing covariate data and maximize the available data for the ROC analysis. Multiple imputation was performed on the predictor dataset with the `mi` command in STATA to generate 35 imputed datasets. A consensus imputed dataset was generated by taking the median (for continuous covariates) or modal (for categorical covariates) values across the 35 imputed datasets. We note that this procedure does not take into account the uncertainty in the consensus imputed estimates, but we reasoned it would be sufficient for obtaining reasonable estimates from the ROC analysis. After confirming that analyses with the consensus imputed dataset yielded results that were consistent with those reported for the analyses described above, we proceeded to

compare the ROC curves of nested models, including a base model that included the base variables controlled for in all analyses (age at study entry, sex, race, and lithium status upon entry into the study) and a full model that included the base variables plus all clinical predictors that were individually associated with treatment failure (see Table 3). The consensus imputed dataset was randomly split into ten non-overlapping subsets of approximately equal size, with approximately the same proportion of censored and event observations across all subsets. Cox models for all four models were then fit using nine out of ten subsets, leaving the tenth subset as a hold-out set. Using the results of the fitted models, linear predictor scores were obtained for observations in the hold-out set. Model fitting and prediction were repeated ten times, where a different subset of data was held out each time. Predicted survival ROC curves over two years were estimated for the linear predictions using the CoxWeights function from the risksetROC R package (12,13). The area under the curve (AUC) for the ROC of the four models were generated, and the differences in AUC among the four were recorded. This process was repeated across 10,000 permutations of survival status and time of censoring pairings. The p-value for AUC difference was derived as the proportion of permuted AUC differences that were greater than the unpermuted AUC difference.

4.4 Results

Figure 1 shows a CONSORT like flow diagram of the study. A total of 345 individuals were enrolled into the study and included in the analysis. Of these, a total of 194 patients successfully advanced to the maintenance phase, while 60 patients failed to remit on lithium monotherapy during stabilization and/or observation phases. Another 91

patients were terminated from the study for other reasons prior to the maintenance phase. Of the 194 patients who entered the maintenance phase, 41 experienced a relapse, 65 were terminated for other reasons, and 88 completed the study or were still in active treatment as of the date of data freeze.

Table 2 shows basic socio-demographic characteristics of the study sample broken down by the final outcome status of the patients, whether they completed the study (or were stabilized in maintenance and still active on the study), experienced a treatment failure, or were terminated for other reasons. There were no significant differences in age, sex or race between these three broad outcomes. Patients who entered the study stable on lithium monotherapy were significantly more likely to complete the study compared with those who either were on lithium and other psychotropic medications or were not on lithium on study entry. There were also significant differences between the sites in the outcomes achieved by the patients. These differences were largely explained by the proportion of patients at each site that entered the study stable on lithium, highlighting the importance of controlling for this potential confounder in subsequent analyses.

We then examined the association between hypothesized clinical predictors of lithium response and treatment response. Table 1 shows the list of clinical predictors that were selected a priori for investigation and the self and clinician rated scales from which they were derived. We examined each predictor individually in survival models controlling for factors that we reasoned may confound the relationship with treatment response because they are important socio-demographic factors or were found to differ with outcome status, including age at study entry, sex, race, and lithium status upon entry into the study. Table

3 shows the results for those clinical predictors that were significantly associated with treatment response at nominal significance of $p < 0.05$.

The significant clinical predictors fell into four main categories: baseline anxiety symptoms, lifetime clinical features, daily functioning, and life events. The severity of anxiety symptoms at baseline as measured by total score on the HAM-A was significantly associated with increased risk of treatment failure when examined as a continuous covariate (hazard ratio [HR] 1.05, 95% confidence interval [CI] 1.03 to 1.08) and categorically as none, mild, moderate and severe (results not shown). Interestingly, a pre-existing diagnosis of co-morbid anxiety disorder meeting DSM-IV criteria was not associated with treatment response, suggesting that baseline symptoms rather than lifetime diagnosis are more relevant. The lifetime clinical features that positively associated with increased risk of treatment failure included a history of migraine (HR 1.62, 95% CI 1.03 to 2.55), suicidal behavior (with an apparent dose-response relationship of HR 1.65, 95% CI 0.95 to 2.86 for ideation and HR 2.03, 95% CI 1.16 to 3.53 for more serious attempts) and history of mixed episodes (HR 1.60, 95% CI 1.01 to 2.53), as well as a chronic (non-episodic) pre-treatment course of illness (HR 2.92, 95% CI 1.76 to 4.83). Overall, functional disability related to illness was also an important predictor of treatment failure as assessed by the clinician with regard to lifetime disability (HR 1.80, 95% CI 1.13 to 2.86) and self-rated current disability on the Sheehan Disability Scale completed at baseline (HR 1.06, 95% CI 1.03 to 1.08). The self-rated current disability measure encompassed functional impairment in work, family and social life, all of which were found to be significantly associated with treatment failure, but for simplicity only results of total impairment are shown. Finally, life adversity in the form of past childhood physical abuse

(HR 1.97, 95% CI 1.28 to 3.03) or recent negative life events (HR 1.02, 95% CI 1.00 to 1.03) as captured by the Childhood Life Events scale or the Lifetime Events Questionnaire, respectively, were also associated with increased risk of treatment failure. Stratification of the survival analyses by study phase (Supplemental Table 1) showed that the association of these clinical predictors with treatment failure did not significantly differ between the stabilization/observation versus maintenance phases (all interaction $P > 0.05$).

To evaluate how well a model that included the significant clinical predictors could predict lithium treatment failure over a two-year period, we carried out an additional ROC analysis (Figure 2). The ROC curve for a full model with all significant clinical predictors plus the base variables had an AUC of 0.74, which was significantly different from the null ($p=0.0001$). This was better than the base model that included only the base variables, which had an AUC of 0.68, although the difference was not significantly different ($p=0.13$).

4.5 Discussion

We report here the first results from the PGBD Study, in which we examine clinical predictors of response to lithium treatment for bipolar disorder (BD). Lithium is a first line treatment for BD and can be remarkably effective in controlling the devastating symptoms of BD. However, it is not effective in everyone and anywhere between 40-50% of patients, or even more depending upon the length of follow-up, may need to switch therapeutic regimens. We identified several clinical markers that are associated with failure to respond to lithium treatment. These include current anxiety symptoms, functional impairments, negative life events and certain lifetime clinical features such as a history of migraine,

suicidal ideation/attempts, and mixed episodes, as well as co-morbid personality disorder and a chronic course of illness. Future validation will be required to confirm whether these clinical markers are associated with treatment failure and whether they can be used clinically to effectively distinguish who will and will not do well on lithium before starting therapy. The particular significance of the present study is that it represents a prospective clinical evaluation of lithium response, in contrast to the extensive literature evaluating lithium response retrospectively.

There is a long history of searching for clinical predictors of response to lithium treatment that can help guide treatment decisions. In 2005, Kleindienst and colleagues (14,15) carried out two comprehensive systematic reviews of predictors of lithium response in which they identified nearly 2,000 studies published between 1966 and 2003 on this topic. In one review, they focused on studies that examined psychosocial and demographic predictors, and identified nine that emerged as consistently associated with lithium response. Four were associated with good response (high social status, social support, good compliance, and “dominance” personality trait), while five were associated with poorer response (stress, high expressed emotion, neurotic personality trait, unemployment, and high number of life events). In the other review, they focused on studies that examined clinical predictors of lithium response and identified five that were consistently associated with lithium response across studies. These included a pattern of mania-depression-interval in bi-phasic episodes (so-called MDI polarity sequence) and older age at onset associated with better response, and high number of hospitalizations, a pattern of depression-mania-interval (i.e., DMI polarity sequence), and continuous cycling associated with poorer response. Both reviews concluded that the effects sizes of these factors on

treatment response were relatively small. In a later review, Tighe and colleagues (16) re-examined the data from Kleindeinst and colleagues along with new data that has since been published and generally re-affirmed the conclusions.

The findings from our study agree with some, but not all, of the conclusions from these recent reviews. Similar to the reviews, we found that poor functioning prior to treatment (as captured by unemployment in previous studies), negative life events, and personality disturbances were associated with poor treatment response. On the other hand, we found no evidence for an association of treatment response with age at onset, number of hospitalizations or rapid cycling. We did not have a direct measure of social status; however, we did have the number of years of education, which is a reasonable proxy for social status, but was not associated with treatment response. We also did not have sufficient data to examine associations with episode pattern, which is a compelling observation that has been implicated by previous studies.

Unique to our study, we found intriguing associations of treatment response with current anxiety symptoms as well a history of migraine, suicidal ideation/attempts, and mixed episodes. The observation that symptom level, but not lifetime diagnosis, of anxiety was associated with treatment response echoes findings from the NIMH Collaborative Depression Study that the severity of anxiety is predictive of long-term morbidity in BD (17). With regard to migraines, it has been shown that the prevalence of migraines in patients with BD is 2–3 times higher than in the overall population. Moreover, antiepileptic drugs, such as valproate, are used to treat migraines whereas lithium has no indication in their prophylactic treatment. Thus, co-morbid migraine could mark an etiologically distinct sub-type of BD that is less responsive to lithium treatment (18). With regard to

suicidal behavior, we observed a “dose-response” relationship between the severity of suicidal behavior and increased risk for treatment failure, which lends further credence to the finding. However, this finding should not be taken as a reason for not prescribing lithium to suicidal patients, because lithium has been shown to be effective in reducing the risk of suicide (19,20), even in people who do not experience full mood stabilization on lithium (21). Interestingly, we found that the association with suicidal behavior was noticeably stronger in the maintenance phase of the study, although a formal interaction test of a difference by study phase was not significant. The interpretation of this finding is unclear and warrants further investigation.

If our findings are validated, they may help complete a clinical picture for the types of patients that do not respond well to lithium treatment and lead to clues about the underlying mechanisms that explain poor response. However, these findings should be interpreted in the light of certain limitations of the study. At least three such limitations merit further consideration. First, a sizable proportion of the patients were withdrawn or terminated from the study for a variety of reasons before the pre-specified endpoints of treatment failure or completion of all visits. The survival analysis assumed the risk of treatment failure for these patients was the same as for those who stayed on the study per protocol. It is possible this assumption was not true, and patients who did not complete the study per protocol did so because they were different somehow and possibly experiencing complications that were a precursor to treatment failure. Consistent with this, we did observe differences in certain baseline characteristics for those who did not complete the study per protocol. These individuals tended to be younger ($p=0.091$), non-Caucasian ($p=0.027$), and not stable on lithium monotherapy upon study entry ($p<0.001$). However,

we carried out two alternative analyses of the data and the findings were remarkably similar, suggesting the findings were robust to assumptions made by the survival analysis.

Second, in order to broaden the available population for study, we included patients who were naïve to lithium as well as those who may have taken lithium in the past or were currently on it. It is likely the response trajectories while on study would be different for these patients. Indeed, over one-quarter of the patients entered the study stable on lithium monotherapy and their treatment outcomes were notably better. To account for these differences, we tightly controlled for lithium status in the analysis, so that inferences about the associations with treatment response would not be confounded by these differences.

Finally, the sample size may not have provided sufficient power to detect significant associations with important clinical predictors with smaller effect sizes. However, we emphasize this is one of the largest prospective studies specifically designed to investigate predictors of lithium response. Indeed, it is the only such study that sought to treat patients with monotherapy in order to more firmly link treatment predictors with lithium response unclouded by the use of other psychotropic medications that are frequently taken by patients with BD. This is a unique and noteworthy strength of this study.

Given the devastating burden of BD, there is considerable motivation to develop more effective strategies for treating the disorder. Lithium is an inexpensive and effective treatment, but it does not work for everyone. It would be of tremendous clinical benefit if we could identify predictors of who will respond to lithium before starting treatment. This study provides new evidence that certain clinical factors could be used to help with such predictions. Interestingly, we found that a model which included these clinical factors could predict lithium treatment failure with an AUC of 0.74 that was significantly better

than the null. The hope is that we will be able to improve upon this by developing more sophisticated prediction models that incorporate both clinical and biological (e.g., neuroimaging, neurophysiology and molecular) markers. This is the goal of the PGBD, and this report is our first step towards this goal.

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Table 1. Clinical predictors examined for association with treatment response

Baseline Symptoms	Comorbidity
Anxiety symptoms ^a	Comorbid alcohol abuse/dependence ^c
Hypermotor activity ^b	Comorbid substance abuse/dependence ^c
Irritability and aggressiveness ^b	Comorbid anxiety disorder ^c
Clinical History	Comorbid personality disorder ^c
Age of onset ^c	Functioning
Chronicity of affective disorder ^c	Disability at baseline: impairment ^f
Chronicity of substance abuse ^c	Disability at baseline: family/home life ^f
History of delusions ^c	Disability at baseline: social life ^f
History of auditory hallucinations ^c	Disability at baseline: total ^f
History of visual hallucinations ^c	Disability at baseline: work/school ^f
History of any hallucinations ^c	Functioning during most severe depression ^c
History of headaches lasting 4 to 72 hours ^d	Functioning during most severe mania ^c
History of migraines ^d	Functioning overall ^c
History of suicidal thought/behavior ^c	Years of education ^c
History of suicide attempt ^c	Marital status ^c
Affective psychosis ^c	Life Events
Independence of psychosis episodes ^c	Childhood life events ^g
Mania type: irritable vs. elated ^c	Childhood physical abuse ^g
Number hospitalizations: inpatient ^c	Life events at last visit: total ^h
Number hospitalizations: inpatient + day ^c	Life events at last visit: negative ^h
Presence of mixed episodes ^c	Life events at last visit: positive ^h
Presence of rapid cycling ^c	Family History
	First degree history completed suicide ⁱ
	First degree history bipolar disorder ⁱ
	First degree history depression ⁱ

^a Hamilton Anxiety Rating Scale (HAM-A) administered by clinician at the baseline visit

^b Clinician-Administered Rating Scale for Mania (CARS-M) administered by clinician at the baseline visit

^c Final Best Estimate form of the DIGS evaluation administered by clinician at the screening visit

^d Migraine Questionnaire self-rated at the screening visit

^e Diagnostic Interview for Genetic Studies (DIGS) administered by clinician at the screening visit

^f Sheehan Disability Scale self-rated at the baseline visit

^g Childhood Life Events scale self-rated at the screening visit

^h Life Events Questionnaire from last study visit

ⁱ Family History scale administered by clinician at screening visit

Table 2. Socio-demographic characteristics of the study sample by final outcome status

	Completed Study ^a (n=88)	Treatment Failure ^b (n=101)	Terminated Other ^c (n=156)	p-value
Age, mean years \pm SD	43.84 \pm 15.48	42.20 \pm 13.32	41.66 \pm 14.60	0.53
Sex, n (%)				
Male	41 (46.59)	51 (50.50)	67 (42.95)	0.49
Female	47 (53.41)	50 (49.50)	89 (57.05)	
Race, n (%)				0.06
Asian	2 (2.27)	1 (0.99)	4 (2.56)	
Black	7 (7.95)	7 (6.93)	28 (17.95)	
White	77 (87.50)	89 (88.12)	115 (73.72)	
More than one race	2 (2.27)	4 (3.96)	9 (5.77)	
Ethnicity, n (%)				0.94
Hispanic	3 (3.41)	3 (3.00) ^d	6 (3.85)	
Non-Hispanic	85 (96.59)	97 (97.00)	150 (96.15)	
Li Status, n (%) ^e				<0.001
Li monotherapy	56 (63.64)	16 (15.84)	25 (16.03)	
Li plus other meds	19 (21.59)	47 (46.53)	58 (37.18)	
Not on Li	13 (14.77)	38 (37.62)	73 (46.79)	
Site, n (%)				0.001
UCSD	7 (7.95)	11 (10.89)	11 (7.05)	
Case Western	10 (11.36)	21 (20.79)	40 (25.64)	
Indiana	8 (9.09)	12 (11.88)	6 (3.85)	
Johns Hopkins	5 (5.68)	8 (7.92)	28 (17.95)	
Bergen	10 (11.36)	8 (7.92)	21 (13.46)	
Chicago	2 (2.27)	4 (3.96)	11 (7.05)	
Iowa	12 (13.64)	9 (8.91)	13 (8.33)	
Michigan	17 (19.32)	14 (13.86)	10 (6.41)	
Penn	4 (4.55)	4 (3.96)	8 (5.13)	
Dalhousie	12 (13.64)	9 (8.91)	5 (3.21)	
Mayo Clinic	1 (1.14)	1 (0.99)	3 (1.92)	

^a Includes subjects who completed all study visits or achieved maintenance and still active on study^b Includes subjects who failed to remit or who relapsed on lithium monotherapy^c Includes subjects who withdrew from the study or were terminated for other reasons^d The ethnicity of one patient was unknown^e Lithium status at study entry; other medications refers to psychotropic medications except benzodiazepines or zolpidem

Table 3. Hazard ratio (HR) associations between clinical predictors and treatment response

	# treatment failures / total person-days ^a	HR (95% CI); p-value
Baseline Anxiety Symptoms (cont.) ^b	98 / 105053	1.05 (1.03 – 1.08); p<0.001
Chronicity of Affective Disorder		
Non-Chronic	32 / 65683	1.00
Chronic	58 / 28693	2.92 (1.76 – 4.83); p<0.001
History of Migraine		
No	71 / 86811	1.00
Yes	28 / 17094	1.62 (1.03 – 2.55); p=0.037
History of Suicidal Behavior		
None	22 / 42134	1.00
Suicidal Ideation	32 / 30103	1.65 (0.95 – 2.86); p=0.077
Suicide Attempt	36 / 22915	2.03 (1.16 – 3.53); p=0.012
History of Mixed Episodes		
No	46 / 65213	1.00
Yes	44 / 28388	1.60 (1.01 – 2.53); p=0.046
Overall Functioning		
Not Disabled	46 / 67890	1.00
Disabled	39 / 24587	1.80 (1.13 – 2.86); p=0.013
Disability at Baseline: Total (cont.) ^b	97 / 102885	1.06 (1.03 – 1.08); p<0.001
Disability at Baseline: Impairment		
No	36 / 68392	1.00
Yes	61 / 35389	1.85 (1.15 – 2.97); p=0.011
Childhood Physical Abuse		
No	61 / 82077	1.00
Yes	37 / 21477	1.97 (1.28 – 3.03); p=0.002
Negative Life Events (cont.) ^b	91 / 94193	1.02 (1.00 – 1.03); p=0.020

^a This is the number of treatment failures defined as failure to remit or a relapse on lithium monotherapy over the total number of days of follow-up from study entry to the last visit for all patients in the specific category; sums of treatment failures and person-days of follow-up may differ across covariates due to missing data.

^b For continuous covariates the number of treatment failures per total person-days of follow-up is shown for all subjects with non-missing data for that covariate

Supplemental Table 1. Hazard ratio (HR) associations between clinical predictors and treatment response over the stabilization and maintenance phases of the study.

	Stabilization Phase		Maintenance Phase		
	Events/ person-days	HR (95% CI)	Events/ person-days	HR (95% CI)	Interaction HR (95% CI)
Baseline Anxiety Symptoms (cont.)	58 / 30230	1.06 (1.03-1.09)	40 / 74823	1.04 (0.99-1.09)	0.98 (0.93-1.03)
Chronicity of Affective Disorder					
Non-Chronic	11 / 14592	Ref	21 / 51091	Ref	
Chronic	43 / 12929	3.96 (1.94-8.12)	15 / 15764	1.83 (0.84-4.00)	0.50 (0.19-1.32)
History of Migraine					
No	40 / 22348	Ref	31 / 64463	Ref	
Yes	18 / 7575	1.37 (0.77-2.42)	10 / 9519	1.80 (0.86-3.74)	1.48 (0.60-3.67)
History of Suicidal Behavior					
None	14 / 9692	Ref	8 / 32442	Ref	
Ideation	19 / 9116	1.37 (0.68-2.77)	13 / 20987	2.55 (1.01-6.39)	1.63 (0.53-5.01)
Attempt	21 / 8848	1.37 (0.69-2.72)	15 / 14067	4.30 (1.70-10.90)	2.35 (0.77-7.11)
History of Mixed Episodes					
No	24 / 15602	Ref	22 / 49611	Ref	
Yes	30 / 11847	1.37 (0.77-2.46)	14 / 16541	1.76 (0.84-3.69)	1.05 (0.44-2.51)
Overall Functioning					
Not Disabled	22 / 16894	Ref	24 / 50996	Ref	
Disabled	29 / 9930	2.49 (1.37-4.52)	10 / 14657	1.47 (0.64-3.34)	0.59 (0.23-1.52)
Disability Baseline: Total (cont.)	58 / 29584	1.06 (1.03-1.09)	39 / 73301	1.05 (1.01-1.10)	0.99 (0.95-1.04)
Disability Baseline: Impairment					
No	14 / 13472	Ref	22 / 54920	Ref	
Yes	44 / 16307	2.00 (1.05-3.80)	17 / 19082	1.53 (0.71-3.29)	0.69 (0.28-1.73)
Childhood Physical Abuse					
No	33 / 21008	Ref	28 / 61069	Ref	
Yes	24 / 8653	1.91 (1.10-3.32)	13 / 12824	1.98 (0.98-3.99)	1.11 (0.47-2.59)
Negative Life Events (cont.)	52 / 26865	1.02 (1.00-1.04)	39 / 67328	1.02 (0.99-1.05)	1.01 (0.98-1.04)

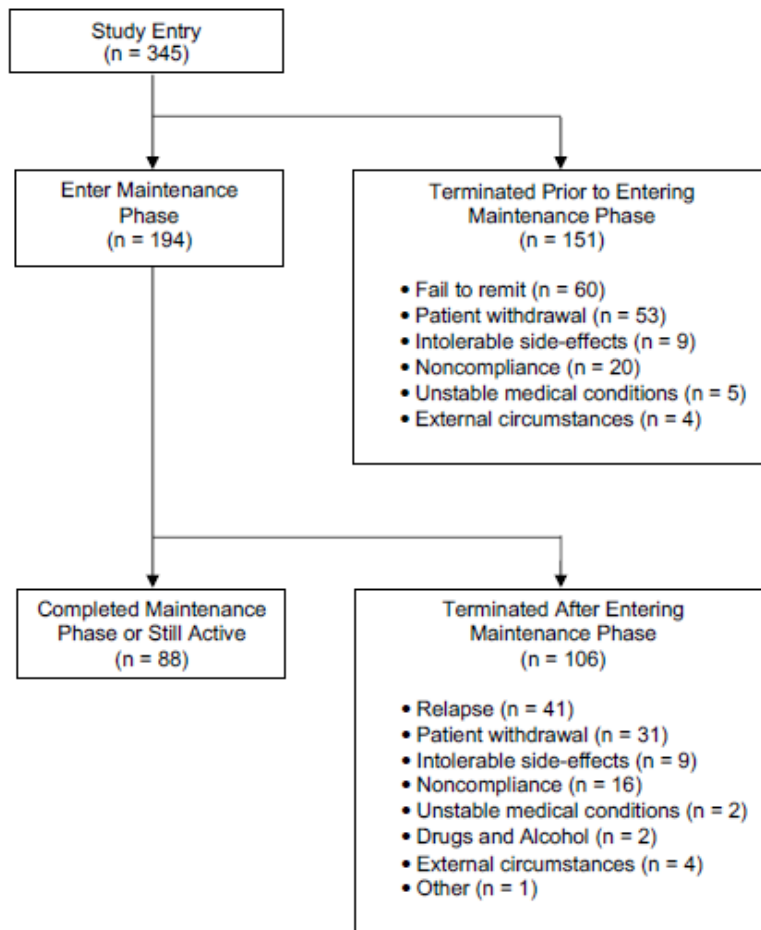


Figure 1. Consort flow diagram of patients in the Pharmacogenetics of Bipolar Disorder Prospective Trial.

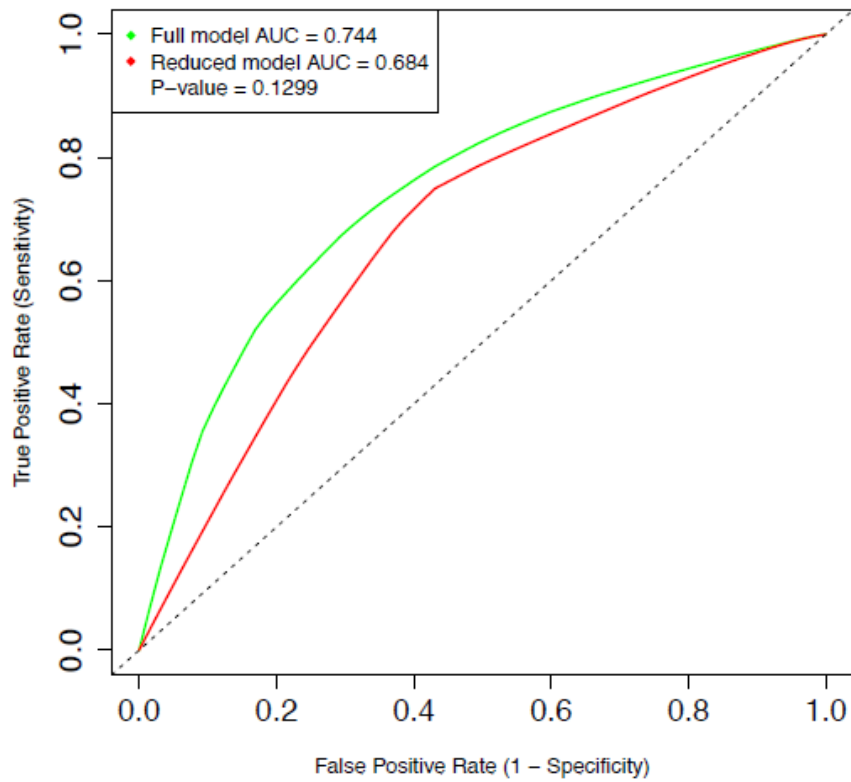


Figure 2. ROC curves for the prediction of lithium treatment failure over a two-year period for a base model that included the base factors controlled for in all analyses (age at study entry, sex, race, and lithium status upon entry into the study) and a full model that included these base factors, all clinical predictors that were individually associated with treatment failure as reported in Table 3.

Chapter 5

Genetic Predictors of Response to Lithium Treatment in The Pharmacogenomics of Bipolar Disorder (PGBD) Study

5.1 Abstract

Background

Given the devastating burden of bipolar disorder (BD), there is considerable motivation to develop effective strategies for treating the disorder. Lithium is an inexpensive and effective option, but it does not work for everyone. This paper seeks to identify genetic predictors of treatment response.

Methods

A multi-site prospective trial of lithium treatment in bipolar 1 disorder was carried out. Patients were gradually discontinued from all other psychotropic medications and stabilized on lithium monotherapy over four months. Once a sustained clinical remission (defined by a score of <3 on the CGI for four weeks) was achieved, patients were followed for up to two years to monitor clinical response. DNA was collected at the baseline and SNP genotyped for subsequent genome-wide association study (GWAS). Lead SNPs from previous studies, genetically imputed gene expression levels using reference data from post-mortem brains samples of the limbic system (i.e., a transcriptome-wide association study or TWAS), and polygenic risk scores for psychotic illnesses were all tested for association with lithium response. Cox proportional hazard models were used to examine the relationship between these genetic predictors and time on study until treatment failure in a final sample of 319.

Results

None of the lead SNPs or imputed gene expression levels were associated with treatment response after correction for multiple testing. However, enrichment analyses of nominally significant genes from the TWAS (n=344 for the amygdala; n=394 for the sACC) identified several significant (FDR<0.05) pathways. Of particular interest were the SET1A (CORUM:6469) and SET1B (CORUM:6470) gene sets and negative regulation of Wnt signaling pathway (GO:0030178). Bipolar PRS were also inversely associated with treatment failure during the stabilization phase (HR: 0.69; 95% CI: 0.48-0.98), but not during the maintenance phase (HR: 1.48; 95% CI: 0.96-2.27).

Conclusions

Despite the relatively small sample size by conventional GWAS standards, we observed several intriguing findings which suggested that genetic variation in histone methylation and Wnt signaling pathways may influence lithium treatment response. Both of these pathways have been implicated previously in lithium's therapeutic mechanisms of action. In addition, there was modest evidence that increasing PRS for bipolar disorder may be associated with better treatment response during treatment stabilization. Larger samples will be needed to confirm these findings and determine whether they may be used clinically to distinguish which patients will do well on lithium before starting therapy.

5.2 Introduction

Bipolar disorder (BD) is a severe mental illness that affects between 1 to 4.4% of the population worldwide, depending on how broadly you define the disorder [1, 2]. It is characterized by an episodic course of illness that alternates between extreme mood states of mania and depression, and it is associated with longstanding disability [3]. It imposes a significant burden on individual sufferers as well as on overall public health. It has been estimated that the costs of care of individuals with bipolar disorder in the US alone exceeds \$45 billion per year [4].

Lithium is recommended as a first-line therapy by established treatment guidelines [5], and it has been reported that approximately 30% of patients treated with it respond well to long-term treatment [6]. However, its use associated with certain side effects [7] that range from less (e.g., tremors, dry mouth, and increased urination) to more serious (e.g., thyroid and kidney toxicities). Lithium has a narrow therapeutic window requiring close monitoring to maintain appropriate levels [8], especially to avoid potentially life-

threatening toxicity. Due to these complications, and the aggressive efforts of pharmaceutical companies to market alternative treatments [9], several other classes of drugs that have shown some efficacy in BD have been increasingly prescribed to treat patients with BD. These include certain anticonvulsants (e.g., valproic acid and lamotrigine) and other second generation antipsychotics (most notably, quetiapine, aripiprazole, and risperidone). As a result, patients often endure an extended period of trial and error to find the right medication, during which time they may continue to suffer needlessly and run the risk of significant adverse consequences, including suicide [10].

There is growing interest in developing biomarkers that can help predict how patients with BD might respond to the different available medications before they initiate treatment so that the right prescriptions can be tailored to the individual patients[11]. With regard to lithium, there would be clear and considerable benefit in being able to identify the sub-set of patients who might respond well to lithium so they could be started on it before trying other medications which may or may not be as effective for them.

There is evidence to suggest that genetics factors help shape response to lithium treatment for BD. It has been reported that patients who respond well to lithium are more likely have a family history of BD compared to poor responders [12], and good responders tend to aggregate within families [13, 14]. Motivated by this evidence, at least five studies using modern genome-wide association study (GWAS) methods have been carried out to identify genetic variants associated with lithium response. These studies have reported mixed findings. They were either retrospective in design, where lithium response was assessed based on assessment of prior treatment history, or they were not specifically

designed to test genetic markers for treatment response. As a result, further study of the genetics of response to lithium treatment is clearly warranted.

This paper reports findings from the analysis of GWAS data from the The Pharmacogenomics of Bipolar Disorder (PGBD) Study. The PGBD was a multi-site, prospective study carried out as part of the Pharmacogenomics Research Network (PGRN) and sponsored by the NIGHMS in collaboration with the NIMH. The goal of the study was specifically to test for genetic markers of response to monotherapy with lithium over two years of follow-up. Because the overall sample size was small relative to current GWAS standards, we focused on tests of lead SNPs implicated by previous GWAS of lithium response, individual genes across the genome using a novel transcriptome-wide association study (TWAS) approach that reduces the multiple testing burden and yields findings that point more directly to biologic mechanisms, and specific polygenic risk scores for psychotic mental illnesses for which lithium may be effective.

5.3 Methods

Study Design

The details of the PGBD Study have been described elsewhere [21]. Briefly, it was a prospective, multi-site study in which patients with BD were stabilized on lithium monotherapy and followed for up to two years or until recurrence of their illness. Patients were enrolled at nine sites in the United States and two internationally. All study procedures were approved by local Institutional Review Boards (IRBs), and all patients provided informed consent.

Patients were included in the study if they: 1) had bipolar I disorder in any phase of illness; 2) were naïve to or not presently on lithium and had at least one affective episode meeting DSM-IV criteria in the last 12 months, or were currently on lithium and did not have any history of mood episodes meeting DSM-IV criteria in the last 6 months; 3) were able to give informed consent; 4) were 18 years or older; and 5) were currently symptomatic, as defined as a CGI-S score of at least 3 (mild severity), unless the patient entered the study already stable on lithium monotherapy. Women of child-bearing potential were included if they agreed to use adequate contraception and inform their doctor at the earliest possible time of their plans to conceive.

Patients were excluded if they: 1) were unwilling or unable to comply with study requirements; 2) had renal impairment (serum creatinine >1.5 mg/dL); 3) had thyroid stimulating hormone (TSH) over >20% above the upper normal limit or, if on thyroid medication, had not been euthyroid for at least 3 months before the first visit; 4) were currently in crisis such that inpatient hospitalization or other crisis management should take priority; 6) met criteria for physical dependence requiring acute detoxification from alcohol, opiates or barbiturates; 7) were pregnant or breastfeeding; 8) had participated in a clinical trial of an investigational drug within the past 1 month, or 9) had a history of lithium toxicity, not due to mismanagement or overdose, that required treatment.

All patients enrolling in the study were either already on lithium monotherapy or they went through a stabilization phase that lasted a maximum of 16 weeks – and included visits every other week for the first 8 weeks and one visit per month for the next two months – during which they were stabilized on lithium monotherapy. They were then observed in an observation phase lasting 4 weeks to confirm they were in remission defined by having

a Clinical Global Impression of Severity Scale (CGI-S) score of <3 (mildly ill) for at least 4 weeks. After the observation phase, the patients entered a two-year maintenance phase, during which they were assessed every 2 months to monitor their on-going clinical response. Throughout the follow-up, patients were allowed to take a benzodiazepine for anxiety and/or zolpidem for sleep.

Clinical Outcomes

Patients were followed until one of three endpoints: 1) they completed all study visits over two years of the maintenance phase (or had achieved the maintenance phase and were still active in the on-going study by the date of the data freeze); 2) they were terminated from the study before completion of all visits because of failure to achieve (i.e., failure to remit) or maintain (i.e. relapse) stabilization on lithium; or 3) they were terminated from the study for other reasons. Failure to remit was defined by the inability to achieve clinically sustained remission (where remission was documented as described above) by the end of the observation phase or based on clinical judgment that the patient was unable to adequately stabilize on lithium monotherapy. Relapse was evaluated using the Mood Episode Checklist which summarizes DSM-IV criteria for mania and depression and was collected at each visit during the maintenance phase. Relapse was defined by the following: 1) met criteria for mania and had a CGI-S of 5 (markedly ill) or greater; 2) met criteria for a major depressive episode with 4 week duration; 3) met criteria for a mixed episode with CGI-S of 5 or greater; 4) psychiatric hospitalization for a mood episode was required; or 5) in the physician's judgment the patient could not be managed on monotherapy and a

change in medication was required. Episodes of hypomania by itself without impairment of function were not considered relapses.

Genetic Data

Blood samples were collected from all patients at the baseline visit for investigation of genetic and other molecular predictors of response to lithium treatment. Samples were genotyped using the Illumina PsychArray for subsequent trans-ethnic GWAS analyses. SNP genotypes were called using standard software, and extensive quality control procedures were carried out following the Psychiatric Genomics Consortium best practices and the RICOPILI pipeline [22]. SNPs and subjects were retained for downstream analyses using the following QC parameters: SNP missingness <0.05 (before subject removal); subject missingness <0.02 ; autosomal heterozygosity deviation ($|F_{het}| < 0.2$); SNP missingness <0.02 (after subject removal); difference in SNP missingness between cases and controls <0.02 ; and SNP HWE ($P > 1e-6$ in controls and $P > 1e-10$ in cases). Subjects were further screened for relatedness to any other subject (closer than 2nd degree, $\pi < 0.2$), unusual homozygosity, sex mismatch, and principal component analysis (PCA) ancestry outliers. Imputation was carried using the pre-phasing/imputation stepwise approach implemented in IMPUTE2 / SHAPEIT (chunk size of 3 Mb and default parameters) with the 1000 Genomes Phase 3 multi-ancestry reference panel [23].

TWAS Data

We examined gene level associations using a transcriptome-wide association study (TWAS) approach to test if genetically determined gene expression levels were associated

with lithium response. The data to impute genetically determined gene expression levels in the PGBD sample came from an RNA-sequencing study of a post-mortem brain collection from the Lieber Institute for Brain Development. This collection includes samples from two brain regions implicated in BD - the amygdala (n=243) and the subgenual anterior cingulate cortex (sACC; n=268). RNA-sequencing of these samples have been described elsewhere (Zandi et al., In Preparation). Briefly, libraries were constructed using Illumina TruSeq Stranded Total RNA Ribo-Zero sample Prep Kit and then sequenced using an Illumina HiSeq 2000. Reads were mapped to the hg38/GRCh38 human reference genome with the splice-aware aligner HISAT2 version 2.0.4. The program featureCounts was then used to derive a read count matrix for gene expressed features based on GENCODE release 25 (GRCh38.p7) annotation. Genome-wide SNP genotype data was generated on these samples using several different Illumina SNP chips, and all samples were subsequently imputed using 1000 Genomes Phase 3 multi-ancestry reference panel.

We analyzed this data to generate predictive models for imputing gene expression levels with SNPs in cis ($\pm 500\text{kb}$) within each gene. We generated two sets of predictive models, one for each brain region, and retained for downstream analyses only those models for genes with significant cis SNP heritability ($p\text{-value} < 0.01$). Building on prior work by Collado-Torres et al. in schizophrenia [24], we employed four FUSION-based methods to generate predictive models controlling for SNP and expression principle components: best linear unbiased prediction (BLUP), elastic net, lasso and top1. SNP weights were constructed from $8124 \text{ (genes)} \times 4 \text{ (FUSION methods)}$ predictive models for sACC and $8039 \text{ (genes)} \times 4 \text{ (FUSION methods)}$ predictive models for amygdala after heritability filtering. For each gene, we selected the optimal model by taking the model that had the

highest 5-fold cross-validated R squared value among the models that have non-zero estimates. For both the amygdala and sACC, BLUP was selected most often for 3014 out of 8039 models and 3142 out of 8124 models, respectively. Elastic net was selected least often for 757 out of 8039 models and 727 out of 8124 models, respectively (Supplemental Figure 1). We used the best predictive model for each gene to impute expression levels in the PGBD sample by taking the inner product of the SNP weights and the individual-level genotype data. The imputed gene expression levels were then used in downstream analyses of the PGBD sample as described below.

Polygenic Risk Scores

Summary statistics of association results from the latest Psychiatric Genomic Consortium (PGC) GWAS of schizophrenia [25] and BD [26] were used to compute polygenic risk scores (PRS) for each patient in the PGBD sample. PRSice was used to explore different p-value thresholds for selecting SNPs from the PGC discovery summary results. Based on an examination of the results across the models tested, we reported results based on a threshold of $p < 0.04$. Given that the PGC GWAS were based almost exclusively on northern European samples, we additionally carried out a sensitivity analysis in which we generated PRS scores just for the Caucasian samples in PGBD using these samples to clump SNPs for selection.

Survival Analysis

We used Cox Proportional Hazard models in a time-to-event framework to analyze the associations between lithium response and genetic predictors, including individual lead

SNPs, imputed gene expression levels, or polygenic risk scores. Time was operationalized as the time from entry into the study until the time to treatment failure - defined as the time of the last visit at which the patient was determined to have failed to remit or to have relapsed - or the time of the last visit in the on-going study if the patient did not experience a treatment failure. Models were tested using time over the entire course of the study and then stratified by time over the stabilization phase (which we defined here as including the observational phase described above) versus the maintenance phase. All models controlled for potential confounders including age at study entry, sex, race, and lithium status upon entry into the study. These variables were selected from the available data because they are important socio-demographic factors that experience indicated may be relevant and/or they were found to differ with treatment outcome. Race was defined based on self-report with the following categories: African American, Asian, Caucasian, more than one race. Self-report was used to be consistent with prior reports of findings from the PGBD, and it was found to map reasonably well on to genetically determined ancestry as visualized by principal component analysis of the SNP genotype data (Supplementary Figure 2).

Pathway Analysis

We performed gProfiler pathway analysis [27] on the nominally significant (p -value < 0.05) results from the TWAS analysis to check for pathway enrichment. gProfiler uses a hypergeometric test to evaluate for enrichment of pathway genes in a user-supplied list of genes. Pathways tested come from GO (including cellular components, molecular functions and biological processes) [28, 29]; the KEGG [30] and Reactome [31] databases; predicted target sites of miRNAs from the miRBase [32]; predicted target sites of

transcription factors from TRANSFAC [33]; information about protein complexes and protein–protein interaction networks from the CORUM database [34] and BioGRID [35]; protein expression data from the Human Protein Atlas [36]; and gene annotations of physiological and disease phenotypes from the Human Phenotype Ontology [37] and Online Mendelian Inheritance in Man (OMIM) resource [38]. We supplied as the background for these enrichment tests the lists of all genes tested in the TWAS separately for the sACC and amygdala.

5.4 Results

We included in the analysis all patients enrolled in the study who completed at least four weeks of follow-up and had evaluable genotype data, which after all QC procedures included 319 patients. Table 1 shows basic socio-demographic characteristics of the study sample broken down by the final outcome status of the patients, that is if they completed the study per protocol, experienced a treatment failure, or were terminated for other reasons. There were no significant differences in age, sex or race between these three broad outcomes. Patients who entered the study stable on lithium monotherapy were significantly more likely to complete the study compared to others. There were also significant differences between the sites in the outcomes achieved by the patients, but these differences were largely explained by the proportion of patients at each site that entered the study stable on lithium, highlighting the importance of controlling for this potential confounder in subsequent analyses.

Lead SNPs

We first tested for associations with seven different lead SNPs that have been implicated by previous GWAS of lithium response. Table 2 shows hazard ratio (HR) associations between these lead SNPs and lithium response in the current study over the full follow-up, and then stratified by the stabilization and maintenance phases of the study. None of the lead SNPs were significant predictors of response over the full follow-up (Table 2). After stratification of the follow-up period, two SNPs located in an intron of *GADL1* from the Chen et al. study [16], rs17026688 and rs17026651, were significantly associated with response during the stabilization phase. However, the minor allele frequencies of these two SNPs were exceedingly rare in our sample such that the tests of associations were highly unstable and, thus, unreliable.

TWAS

We next carried out gene level tests in which we examined the associations between genetically determined gene expression levels and response to lithium treatment. We used as the reference for these analyses one of the largest available post-mortem brain samples with data on two brain regions that have been implicated in BD, the amygdala and the subgenual anterior cingulate cortex (sACC).

Examination of Q-Q plots for the gene level association results suggested there was no inflation of test statistics (Supplementary Figure 3). If anything, there was some deflation, likely due to the small sample that was under-powered. Figure 1 shows Manhattan plots of the results for the full follow-up, as well as the stabilization and maintenance phases. No genes were significantly associated with lithium response over the entire follow-up for imputed levels from the amygdala or the sACC after genome-wide

correction. A total of 344 genes were nominally significant from the amygdala, and 394 genes were nominally significant in the sACC. Of these, there were only 7 genes that were in common between the two brain regions, including *PSMC3*, *ACO93838.4*, *CCDC88B*, *CSF1*, *BCL11B*, *Y_RNA*, and *MAD2L2*.

Stratification by study phase (stabilization vs maintenance) also did not yield any genome-wide significant findings. Of the genes nominally associated with failure to remit in the stabilization phase or relapse in the maintenance phase, 17 and 14 genes were in common for amygdala and sACC, respectively. In addition, 390 (amygdala) and 380 (sACC) genes were unique to stabilization phase and 334 (amygdala) and 378 (sACC) genes were unique to maintenance phase.

Pathway analysis

To infer the potential biological processes underlying the TWAS associations with lithium response, we inputted the nominally significant genes for the amygdala and sACC respectively into gProfiler for functional profiling. For these analyses, we only considered the results from full study follow-up. Interestingly, several distinct sets of pathways were significantly enriched ($FDR < 0.05$) in the associated genes across the two brain regions (Table 3 and Figure 2). For the amygdala, there was a cluster of enriched pathways from the CORUM database, which captures information about protein complexes and protein–protein interaction networks. A common theme for these enriched pathways was they included protein complexes involved in histone methyltransferase and acetyltransferase activity. Prominent among these were the SETD1A, SETD1B, and WRAD complexes, which contain proteins that are involved specifically in H3K4 methyltransferase activity

and either directly or indirectly involve SETD1A which has previously been associated with schizophrenia [39]. For the sACC, several GO biological process pathways were also enriched revolving around cellular response to stimuli (GO:0051716, GO:0050896, GO:0048584); signaling and signal transduction (GO:0023052, GO:0007165, GO:0007166); and particularly Wnt signaling (GO:0030178).

Polygenic Risk Scores

Finally, we tested for associations with polygenic risk scores derived for severe psychotic mental illnesses, including bipolar disorder and schizophrenia, which we hypothesized may influence treatment response to lithium. Neither of the PRS for BD nor schizophrenia were significantly associated with lithium response over the full study follow-up (Table 4). However, increasing PRS for BD was inversely associated with failure to remit during the stabilization phase (hazard ratio [HR]: 0.69; 95% confidence interval [CI]: 0.48-0.98). The inverse association remained significant when we restricted the analysis to patients of European ancestry (HR: 0.76; 95% CI: 0.58-1.00) in a sensitivity analysis. Increasing PRS for schizophrenia was also inversely associated with failure to remit during the stabilization phase, but this was not significant (HR: 0.54; 95% CI: 0.28-1.05)

5.5 Discussion

In this study, we aimed to identify genetic predictors of response to lithium treatment for BD using GWAS data from the PGBD. This is one of the few, if only,

existing studies to test for such associations using a prospective study design in which the goal was to treat patients with lithium monotherapy in order to isolate effects specific to this treatment. Due to the relatively small sample size for GWAS, we decided to focus on tests of lead SNPs that have been reported by previous studies, as well as more global tests of individual genes using TWAS and genome-wide polygenic risk for severe mental illnesses that we hypothesized may be related to treatment response. Two key findings emerged from these analyses. First, although there were no genome-wide significant findings with individual genes using TWAS, several intriguing pathways were found to be enriched among the top results. These included pathways related to histone methylation and acetylation and Wnt signaling, which have been implicated previously in lithium mechanisms of action. Second, we found that increasing polygenic risk for BD was associated with lower risk of treatment failure, at least over an initial stabilization phase of treatment. These findings warrant further investigation both as clues to the mechanisms that may underlie treatment effectiveness with lithium in BD and as potential clinical predictors of treatment response.

At least five GWAS of lithium treatment response in BD have been previously reported. In 2009, Perlis and colleagues conducted a GWAS of mood disorder recurrence among 458 lithium treated patients with bipolar I (BDI) and bipolar II (BDII) disorders from the STEP-BD cohort and a replication sample of 359 BD patients. There were no genome-wide significant results, but the most compelling finding spanned *GRIA2*, an AMPA receptor involved in glutamatergic signalling [15]. In 2014, Chen and colleagues reported results from a GWAS on a subset of 294 BDI patients on lithium treatment from a sample of 1,761 patients of Han Chinese descent recruited by the Taiwan Bipolar

Consortium. Two SNPs in high linkage disequilibrium located in the introns of *GADL1* showed the strongest associations and reportedly had a sensitivity of 93% for predicting lithium response [16]. In 2015, Song and colleagues reported a genetic variant in *SESTD1* associated with lithium-responsive BD from a GWAS comparing lithium responders with healthy controls from Sweden and the UK, including 1,639 self-reported responders versus 8899 controls and 323 clinically-documented responders versus 6,684 controls [17]. Finally, in 2016 and 2018, there were two reports from the Consortium on Lithium Genetics (ConLiGen) which conducted GWAS with 2,653 BD patients retrospectively characterized for lithium response using the Alda Scale [18]. These studies reported finding associations with a locus that contains two long non-coding RNAs (lncRNAs) genes, AL157359.3 and AL157359.4 [19], as well as an inverse association between increasing polygenic risk scores for schizophrenia and poor response to lithium [20].

We were unable to credibly replicate any of these prior associations, although the sample size may have limited our power to detect these associations. This was especially true for the findings from the Chen et al. [16] study in an Asian sample, as the minor allele frequencies of the implicated SNPs from these samples were very rare in the PGBD sample, which was mostly Caucasian and African American. Because the minor allele frequencies were so low, the estimated associations were highly unstable and, therefore, not reliable. Intriguingly, there were trends of inverse associations between SNPs reported by Song et al. [17] and Hou et al. [19] studies, especially during the stabilization phase of treatment, but these findings were not significant and cannot be considered a replication.

Challenges with interpretation of GWAS findings have motivated the development of methods to prioritize causal genes at GWAS loci, including TWAS, which seeks to

identify associated genes by testing variability in genetically determined gene expression. Despite recently suggested vulnerabilities [40], this approach aims to identify associated features underlying lithium response directly, rather than single nucleotide variants with often ambiguous or uncertain annotation and functionality. The TWAS approach may also increase the power over traditional GWAS to identify genetic associations by reducing the burden of multiple testing.

In this study, we used one of the largest available samples of post-mortem brains from the Lieber Institute collection to generate more powerful gene expression prediction models from amygdala and sACC. In the healthy brain, the performance of emotional tasks recruits the limbic neural system, which is comprised of the orbital and medial parts of the prefrontal cortex, including the sACC, and subcortical structures, including the amygdala and ventral striatum [41, 42]. For BD, we have seen consistent findings in the neuroimaging literature that allude to an etiological model in which the abnormalities in the structure and function of the amygdala play a role but also depend on the failure of prefrontal cortical regions to modulate activity [43]. Structural brain abnormalities in the prefrontal cortex have also been confirmed in postmortem studies and with structural MRI [44]. The sACC, as an example, was reduced in volume in patients with BD with a family history of affective disorder [45, 46]. These imply the involvement of complex neurocircuitry in BD neuropathophysiology. It has been reported that BP patients with previous lithium treatment had greater hippocampal and amygdalar volumes than those without lithium exposure [47]. Lithium has also been proposed to have neuroprotective effects leading to the sparing of sACC volume [45]. It is these converging lines of evidence

that motivated us to use data from the amygdala and sACC as our reference for the TWAS of lithium response in the PGBD.

None of the individual gene tests were significant after correction for multiple testing for either the amygdala or the sACC. This is perhaps not surprising, again because of the relatively small sample size. Nevertheless, we did observe significant enrichment of several intriguing gene sets among the top findings. These included genes encoding H3K4 methyl and acetyltransferase complexes as well as those involved in Wnt signaling pathways. Interestingly, the histone H3K4 methylation GO pathway (GO:51568) emerged as the most significant enriched pathway in bipolar disorder, and the only one that was significant after correcting for multiple testing, from a recent cross-disorder pathway analysis of existing GWAS data from the Psychiatric Genomics Consortium (PGC) [48]. In addition, a key gene of the H3K4 methyl and acetyltransferase complexes is SETD1A which has been associated with schizophrenia in recent sequencing studies [39]. Methylation and acetylation of histone proteins are well-established epigenetic changes involved in chromatin modification, especially at the transcription start sites of active genes. Lithium has been found to induce epigenetic changes through different mechanisms, including DNA demethylation and histones acetylation [49]. Moreover, histone deacetylase (HDAC), an enzyme involved in histone deacetylation, is recognized as the primary target of valproic acid, which also has efficacy as a mood stabilizer [50]. Thus, there is evidence that lithium's mechanism of action may, at least partially, be mediated via chromatin remodeling, and the current findings suggest the possibility that genetically determined transcriptional levels of genes involved in chromatin modifications may influence the efficacy of lithium.

Canonical Wnt signaling may also be crucial to lithium response in bipolar disorder [51, 52]. Evidence has shown that lithium action in bipolar disorder may involve both direct and indirect inhibition of glycogen synthase kinase-3 beta (GSK3B) (eg. [53, 54]). GSK3B is a serine-threonine kinase belonging to the glycogen synthase kinase subfamily and is a key protein in the Wnt signaling pathway. Previous studies have reported that the therapeutic sensitivity to lithium may be associated with polymorphisms in the GSK3 β promoter region [55]. In line with this, the current findings suggest that genetic variation determining transcriptional levels of genes involved more broadly in Wnt signaling may further influence lithium treatment response. While intriguing, more research is needed to further investigate the role in moderating responses to lithium treatment of variation in genes involved in both H3K4 methylation and Wnt signaling.

Finally, we tested whether genomewide PRS for psychotic disorders may also predict response to lithium treatment. We found that increasing PRS for bipolar disorder was inversely associated with treatment failure, at least during the stabilization phase. In other words, patients who had greater genetic risk for bipolar disorder appeared to do better on lithium in the short-term when initiating treatment. This finding is in line with previous studies which have shown that patients with a family history of bipolar disorder respond better to lithium [12], and it is consistent with the notion that lithium is most effective for patients with a more classic form of bipolar disorder. We also observed an inverse association between increasing PRS for schizophrenia and treatment failure during the stabilization phase, but this association was not statistically significant. It is also inconsistent with a recent study from ConLiGen, which found that increasing polygenic risk for schizophrenia was significantly associated with worse treatment response [20].

More work is clearly needed to further clarify these conflicting findings. All of the existing studies are probably under-powered and larger sample sizes are needed to evaluate if PRS for bipolar disorder and schizophrenia can predict how patients will respond to treatment. In addition, future work is needed to determine if PRS for lithium response may also be useful for treatment prediction. Reasonably powered PRS for lithium response are still not yet available, but we anticipate they will be of greater interest when they do become available.

This study had several notable strengths. Chief among them is the fact that it is one of the few studies to attempt to follow patients prospectively on lithium monotherapy [21]. Patients with bipolar disorder are typically treated with multiple medications (see Chapter 3), and as a result it can be difficult to tease apart the specific effects of individual medications on clinical outcomes. By following patients on monotherapy, this allowed us to test genetic predictors of treatment response specifically to lithium unconfounded by the use of other medications. In addition, we used a novel TWAS approach that to our knowledge has not been used previously to examine genome-wide predictors of lithium response. Moreover, for this analysis, we were able to use for our reference one of the largest available post-mortem brain samples from regions of the limbic system which prior evidence suggests is especially relevant to the pathology in bipolar disorder.

The study also had several important weaknesses. Following patients on monotherapy is practically very challenging [21]. As a result, there was considerable loss to follow-up over the full course of the study (45.2%). Our analysis assumed that the risk for treatment failure was the same for those patients lost to follow-up as those who stayed on the study. This is a reasonable assumption, but it is one that may be violated as those

who are lost to follow-up might be more likely to go on to switch treatments. In addition, the challenging nature of the study made it challenging to enroll large numbers of patients that are typically needed for GWAS. As a result, the study is likely under-powered to detect most genetic effects on treatment response.

Despite the challenges, we were able to identify some evidence of genetic associations with lithium response that merit further investigation. Our study is among the first results from the PGBD trial in which we examined gene predictors of lithium response for patients with BD. For those who can be effectively treated by lithium, it can be remarkably effective in controlling the devastating symptoms of BD. For the more than 40-50% of patients who do not response well, however, they may need to switch therapeutic regimens mid-treatment. For either groups of patients, knowing beforehand whether or not they will respond well before starting treatment would offer tremendous clinical benefits. Our study serves as one of the on-going attempts to identify predictors of response and realize the potential of personalize medicine.

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Table 1. Socio-demographic characteristics of the study sample by final outcome status

	Completed Study ⁱ (n=85)	Treatment Failure ⁱⁱ (n=97)	Terminated Other ⁱⁱⁱ (n=137)	p-value
Age, mean years \pm SD	44.09 \pm 15.55	42.85 \pm 13.07	41.34 \pm 14.64	0.375
Sex, n (%)				
Male	40 (47.06)	47 (48.45)	61 (44.53)	0.830
Female	45 (52.94)	50 (51.55)	76 (55.47)	
Race, n (%)				0.236
African American	6 (7.06)	7 (7.22)	21 (15.33)	
Asian	2 (2.35)	1 (1.03)	4 (2.92)	
Caucasian	75 (88.24)	85 (87.63)	105 (76.64)	
More than one race	2 (2.35)	4 (4.12)	7 (5.11)	
Ethnicity, n (%)				0.840
Hispanic	3 (3.53)	2 (2.08) ^{iv}	4 (2.92)	
Non-Hispanic	82 (96.47)	94 (97.92)	133 (97.08)	
Li Status, n (%) ^v				<0.001
Li monotherapy	55 (64.71)	15 (15.46)	23 (16.79)	
Li plus other meds	19 (22.35)	44 (45.36)	54 (39.42)	
Not on Li	11 (12.94)	38 (39.18)	60 (43.80)	
Site, n (%)				0.001
UCSD	7 (8.24)	11 (11.34)	10 (7.30)	
Case Western	8 (9.41)	21 (21.65)	28 (20.44)	
Indiana	8 (9.41)	12 (12.37)	6 (4.38)	
Johns Hopkins	5 (5.88)	7 (7.22)	28 (20.44)	
Bergen	9 (10.59)	8 (8.25)	17 (12.41)	
Chicago	2 (2.35)	4 (4.12)	11 (8.03)	
Iowa	12 (14.12)	9 (9.28)	12 (8.76)	
Michigan	17 (20.00)	14 (14.43)	10 (7.30)	
Penn	4 (4.71)	4 (4.12)	7 (5.11)	
Dalhousie	12 (14.12)	6 (6.19)	5 (3.65)	
Mayo Clinic	1 (1.18)	1 (1.03)	3 (2.19)	

i Includes subjects who completed all study visits or achieved maintenance and still active on study

ii Includes subjects who failed to remit or who relapsed on lithium monotherapy

iii Includes subjects who withdrew from the study or were terminated for other reasons

iv The ethnicity of one patient was unknown

v Lithium status at study entry; other medications refers to psychotropic medications except benzodiazepines or zolpidem

Table 2. Hazard ratio (HR) associations between lead SNPs and lithium treatment response

Study	Lead SNPs (MA, MAF)	Full Follow-up (N=319)	Stabilization Phase (N=319)	Maintenance Phase (N=187)
		HR (95% CI); p-value	HR (95% CI); p-value	HR (95% CI); p-value
Perlis (2009)	rs10795189 (G, 0.134)	1.00 (0.63-1.60); 0.98	0.98 (0.55-1.79); 0.97	1.15 (0.55-2.39); 0.72
Chen (2014)	rs17026688 (T, 0.003)	5.07 (0.53-48.33); 0.20	17.72 (1.04-301.62); 0.05	NA
	rs17026651 (G, 0.003)	5.07 (0.53-48.33); 0.16	17.72 (1.04-301.62); 0.05	NA
Song (2016)	rs116323614 (A, 0.030)	0.63 (0.25-1.60); 0.34	0.25 (0.03-1.86); 0.18	1.51 (0.48-4.72); 0.48
Hou (2016)	rs79663003 (C, 0.041)	0.74 (0.33-1.65); 0.46	0.41 (0.10-1.72); 0.22	1.19 (0.43-3.28); 0.74
	rs78015114 (C, 0.041)	0.74 (0.33-1.65); 0.46	0.41 (0.10-1.72); 0.22	1.19 (0.43-3.28); 0.74
	rs74795342 (A, 0.038)	0.53 (0.21-1.33); 0.18	0.41 (0.10-1.74); 0.23	0.68 (0.20-2.35); 0.54
	rs75222709 (G, 0.038)	0.52 (0.21-1.33); 0.18	0.41 (0.10-1.73); 0.22	0.68 (0.20-2.35); 0.54

MA = minor allele, MAF=minor allele frequency in study sample, NA = not available due to small sample size

Table 3. Functional profiling of nominally significant genes (p-value < 0.05) from TWASⁱ

Brain region	Source	Term name	Term id	Term size	Adjusted p-value
amygdala	MIRNA	hsa-miR-548at-3p	MIRNA:hsa-miR-548at-3p	21	0.03
	MIRNA	hsa-miR-548ay-3p	MIRNA:hsa-miR-548ay-3p	21	0.03
	CORUM	Set1A complex	CORUM:6469	3	0.02
	CORUM	Set1B complex	CORUM:6470	4	0.04
	CORUM	Menin-associated histone methyltransferase complex	CORUM:1254	2	0.05
	CORUM	Set1B complex	CORUM:2730	2	0.05
	CORUM	Set1A complex	CORUM:2731	2	0.05
	CORUM	PTIP-HMT complex	CORUM:5195	2	0.05
	CORUM	MLL3 complex	CORUM:6461	2	0.05
	CORUM	WRAD complex (WDR5, RBBP5, ASH2L, DPY30)	CORUM:6849	2	0.05
	CORUM	NSL complex	CORUM:7221	2	0.05
sACC	GO:BP	cellular response to stimulus	GO:0051716	1635	0.01
	GO:BP	cell communication	GO:0007154	1411	0.02
	GO:BP	signal transduction	GO:0007165	1294	0.02
	GO:BP	signaling	GO:0023052	1416	0.02
	GO:BP	positive regulation of biological process	GO:0048518	1423	0.02
	GO:BP	response to stimulus	GO:0050896	1949	0.02
	GO:BP	cell surface receptor signaling pathway	GO:0007166	689	0.02
	GO:BP	positive regulation of response to stimulus	GO:0048584	560	0.02
	GO:BP	positive regulation of cellular process	GO:0048522	1268	0.02
	GO:BP	negative regulation of Wnt signaling pathway	GO:0030178	61	0.05

MIRNA = miRTarBase, CORUM = CORUM protein complexes, GO:BP = GO biological process

ⁱ Table includes pathways with FDR adjusted p-value < 0.05

Table 4. Hazard ratio (HR) associations between standardized PRS predictors and lithium treatment response

	Full Follow-up		Stabilization Phase		Maintenance Phase		Interaction
	Events / PD	HR (95% CI); p-value	Events / PD	HR (95% CI); p-value	Events / PD	HR (95% CI); p-value	HR (95% CI); p-value
Bipolar Disorder PRS							
All Samples	97 / 104953	0.97 (0.74-1.28); p=0.85	58 / 29752	0.69 (0.48-0.98); p= 0.04	39 / 75201	1.48 (0.96-2.27); p=0.08	1.23 (0.81-1.87); p=0.34
European	82 / 84543	0.94 (0.76-1.15); p=0.52	47 / 23375	0.76 (0.58-1.00); p=0.05	35 / 61168	1.21 (0.86-1.70); p=0.27	1.56 (1.02-2.40); p=0.04
Schizophrenia PRS							
All Samples	97 / 104953	0.94 (0.59-1.50); p=0.80	58 / 29752	0.54 (0.28-1.05); p=0.07	39 / 75201	1.77 (0.88-3.56); p=0.11	0.93 (0.59-1.46); p=0.75
European	82 / 84543	0.88 (0.70-1.10); p=0.25	47 / 23375	0.76 (0.56-1.04); p=0.08	35 / 61168	0.96 (0.68-1.36); p=0.83	1.24 (0.79-1.94); p=0.34

PD=person-days

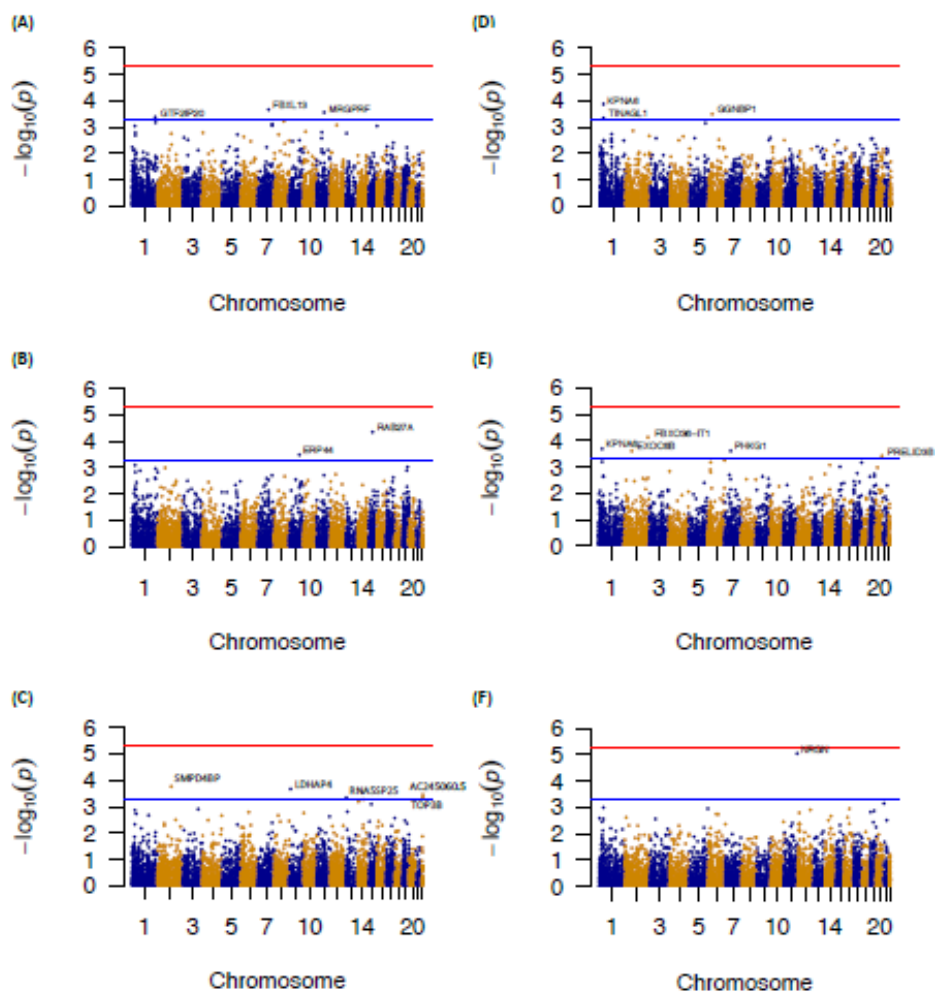


Figure 1. Manhattan plots. Red line is the 5×10^{-6} significance line; blue line is the 5×10^{-4} suggestive significance line. Genes with p-values < 0.0005 are annotated. (A)(B)(C) amygdala full follow-up, stabilization phase, and maintenance phase; (D)(E)(F) sACC full follow-up, stabilization phase, and maintenance phase.

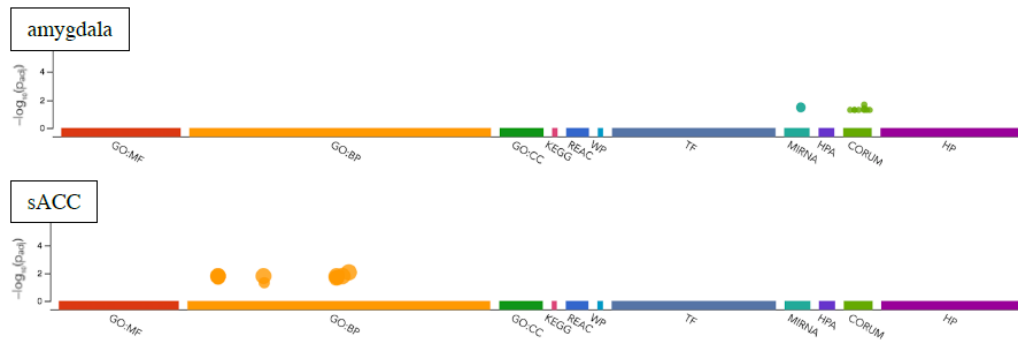
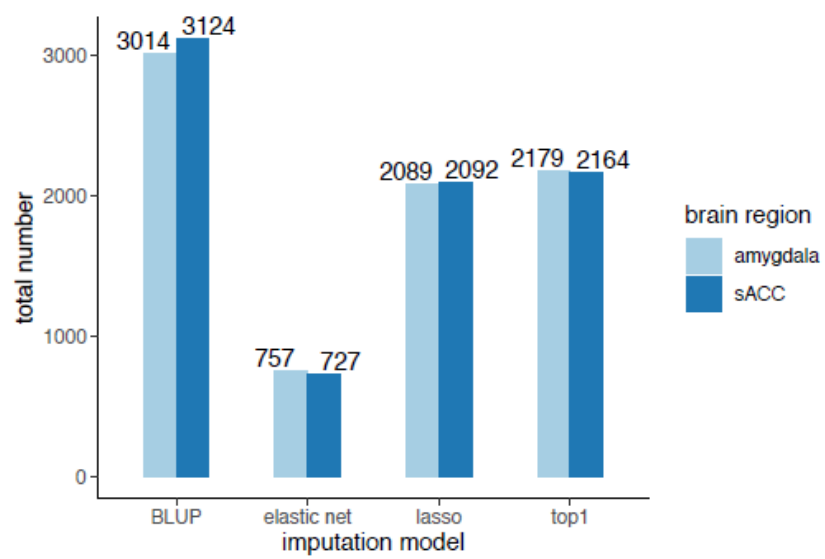
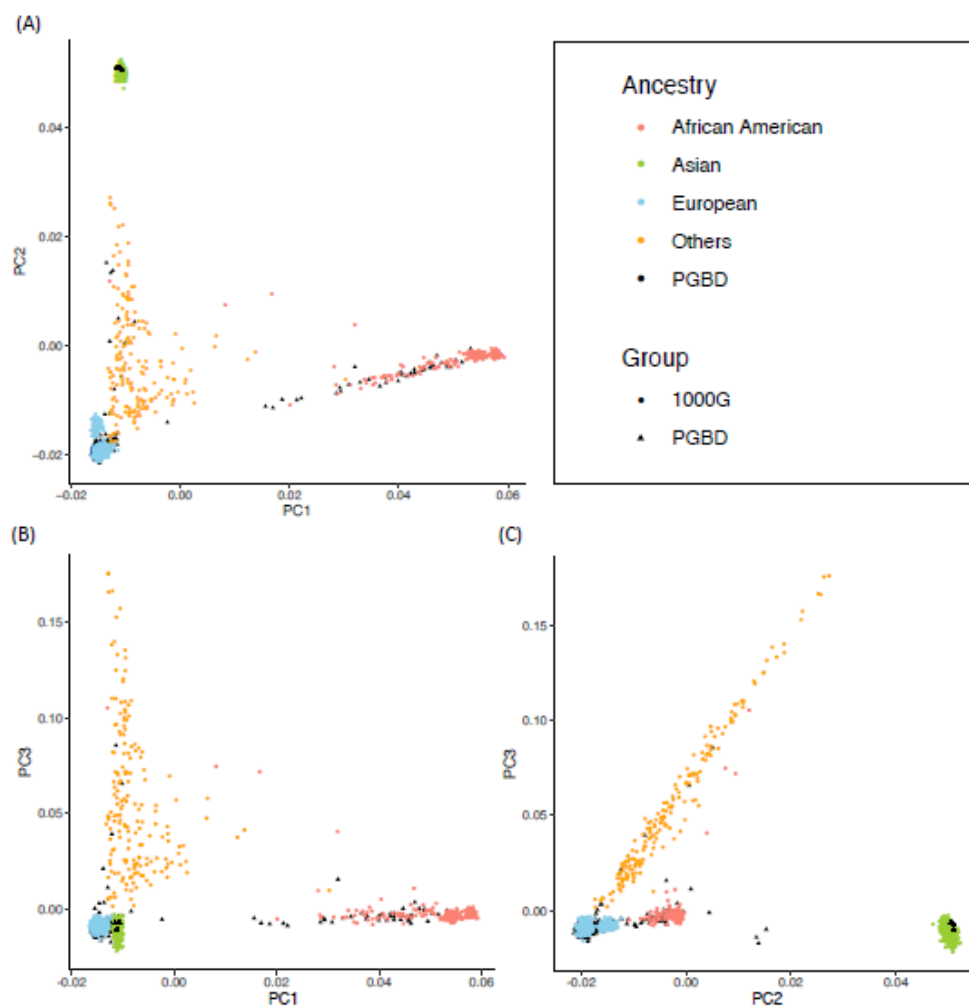


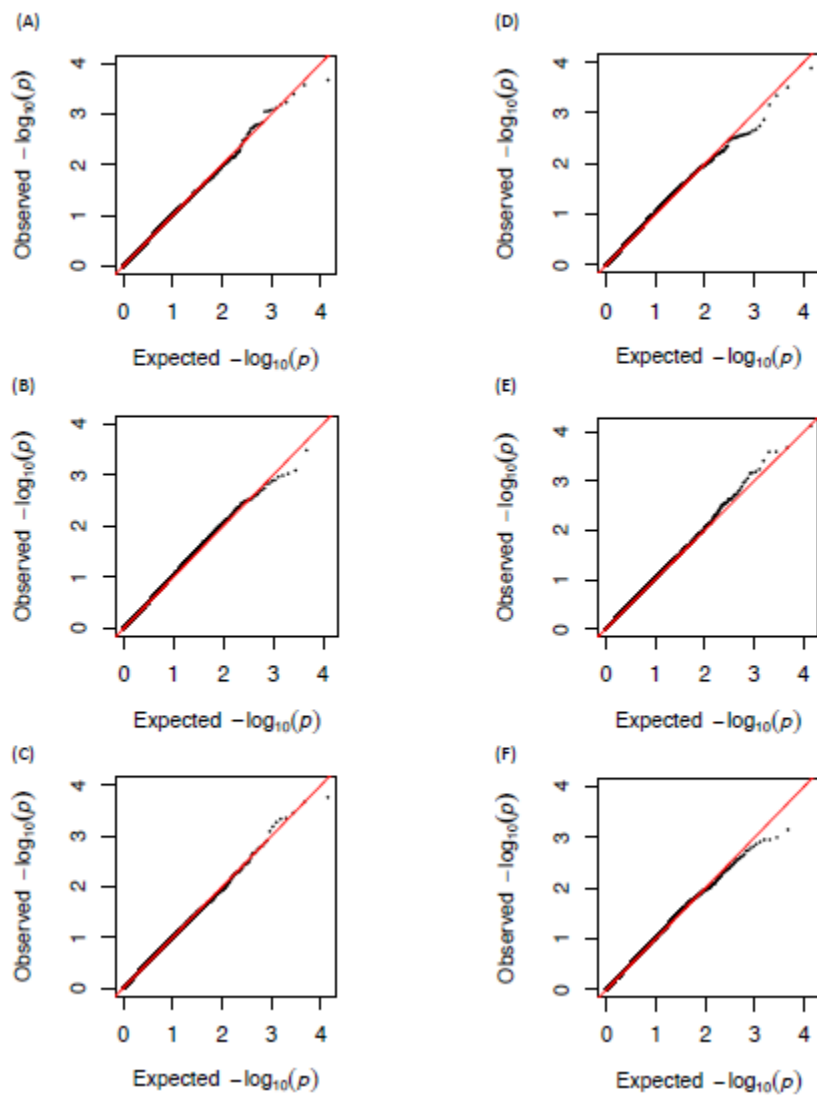
Figure 2. gProfiler pathway analysis visualization from the nominally significant ($p\text{-value} < 0.05$) genes from survival analysis on the full follow-up. Source ID annotation (Raudvere et al. 2019): GO:MF=molecular function; GO:BP=biological process; GO:CC=cellular component; KEGG=Kyoto Encyclopedia of Genes and Genomes; REAC=Reactome; WP=WikiPathways; TF=TRANSFAC; MIRNA=miRbase; HPA=Human Protein Atlas; CORUM=CORUM protein complexes; HP=Human Phenotype Ontology. Significant pathways (FDR adjusted $p\text{-value} < 0.05$) are listed in Table 4.



Supplemental Figure 1. Summary of optimal TWAS imputation model selection.



Supplemental Figure 2. PCA plots for comparing PGBD sample to 1000G ancestries. (A) PC1 vs PC2; (B) PC1 vs PC3; (C) PC2 vs PC3.



Supplemental Figure 3. Q-Q plots.

(A)(B)(C) amygdala full follow-up, stabilization phase, and maintenance phase;
 (D)(E)(F) sACC full follow-up, stabilization phase, and maintenance phase.

Chapter 6

Discussion

There is a long history of searching for predictors of lithium response that can help guide treatment decisions for patients with bipolar disorder. Lithium is one of the most effective treatments for bipolar disorder and it is typically recommended as a first-line treatment [46]. However, only about 30% of patients respond well to lithium [47], while other patients typically need to switch treatments several times in order to find more effective medications, during which time they continue to suffer. As a result, there is considerable motivation to identify predictors of who will respond well so that they can be started right away on lithium, while others could be started on other treatments that are more appropriate for them. This is the promise of so-called precision medicine. My thesis

aims to study trends in the use of lithium and identify clinical and genetic predictors of treatment response to help achieve the goals of precision medicine for patients with bipolar disorder.

In 2005, two comprehensive systematic reviews of clinical predictors of lithium response were carried out by Kleindienst and colleagues in which they identified nearly 2,000 studies published between 1966 and 2003 on this topic [17, 18]. Nearly a decade later, Tighe and colleagues re-examined the data from Kleindeinst and colleagues along with new data that had since been published and generally re-affirmed the conclusions [19]. They identified several demographic, psychosocial and clinical predictors that appeared to be associated with response across the different studies, but the results were not always consistent and the observed effect sizes tended to be relatively small. Few of the previous studies used a prospective designed to investigate predictors of lithium treatment, and none examined predictors of response associated with monotherapy.

There has also been considerable interest in identifying genetic predictors of lithium response. Lithium response tends to run in families, and to date five genomewide association studies (GWAS) of lithium response in bipolar disorder have been conducted. These include a study in 2009 of 458 patients with bipolar I/bipolar II disorders from the STEP-BD cohort [42]; a study in 2014 on a sample of 294 patients with bipolar I disorder on lithium treatment who were of Han Chinese descent recruited by the Taiwan Bipolar Consortium [21], a study in 2015 of 1639 self-reported responders and 8899 controls, as well as 323 clinically-documented responders and 6684 controls, from Sweden and the UK [22]; and two reports from the ConLiGen Consortium which has gathered lithium treatment response data on 2586 patients from around the world [8] [41]. The findings from these

studies have been largely mixed, motivating further efforts to study the genetic contribution to lithium treatment response. Taking the road of the advancement of our understanding of factors that predict lithium response, my thesis work aimed to be a small step that contributes to the goal of precision medicine for BD.

Chapter 3 of this thesis characterizes trends in lithium utilization for the treatment of BD in the U.S. from 1996 to 2015. In this study, we examined trends in reports of lithium and other medications for BD in a representative sample from the National Ambulatory Medical Care Survey (NAMCS), a well-characterized national survey of US office-based practices of physicians from different specialties, conducted annually across a broad range of office settings. Specifically, we examined trends in reports of lithium, mood-stabilizing anticonvulsants, second generation antipsychotics (SGAs) and antidepressants overall and in combinations in visits with a diagnosis of BD. This study further documents the declining reports of using lithium for BD, and corresponding increase in reports of using anticonvulsants and SGAs, despite the fact that lithium is typically recommended as a first line therapy for BD. One of the strengths of this study is that we used data from a nationally representative sample of office based clinical visits across the US. In addition, these data cover a twenty-year period from 1996-2015 and were collected using largely consistent procedures. This allowed us to have a relatively large sample to make statistical inferences, which is an advantage over past BD studies, but it may not be sufficiently large to detect trends in subgroups of patients. By combining the data into two-year intervals, we attempted to obtain more stable results while not losing the ability to observe more fine scale trends, though there is likely still limited power to look at results stratified by visit type or individual medications. Apart from sample size,

the cross-sectional nature of the NAMCS data is another limitation that makes it impossible to assess the prescribing patterns within individuals longitudinally, which is a feature worth looking into when selecting datasets for describing medication prescribing trends.

Chapter 4 of this thesis evaluates clinical predictors associated with response to lithium treatment over time. We analyzed data from the Pharmacogenomics of Bipolar Disorder (PGBD) Study (www.clinicaltrials.gov, NCT01272531), which is one of the first prospective studies of lithium treatment designed to prospectively identify clinical and molecular predictors of lithium response. Specifically, we examined the relationship between clinical measures and time on study until failure to remit or relapse. An advantage of this study is the prospective design of the PGBD trial, which is rare in the previous literature. This is one of the few prospective studies explicitly designed a priori to examine the prediction of lithium response by following patients in lithium monotherapy for up to two years. Unique to our study, current anxiety symptoms, a history of migraine, suicidal ideation/attempts, and mixed episodes were associated with failure to respond to lithium. Future validation is needed to confirm these clinical predictors of treatment failure and their use clinically to distinguish who will do well on lithium before starting therapy. The findings should be seen in the light of the limitations that include a sizable proportion of pre-completion withdrawals or terminations, a diversity in lithium status upon entry (though controlled for in all analyses) and a relatively small sample size.

Chapter 5 evaluates the genetics predictors of lithium response in the form of lead SNPs that have been reported by previous studies, as well as individual genes using TWAS and genome-wide polygenic risk for severe mental illnesses that we hypothesized may be related to treatment response. Although there were no genome-wide significant findings

with individual genes using TWAS, several intriguing pathways were found to be enriched among the top results, including pathways related to histone methylation and acetylation and Wnt signaling that are previously implicated in lithium mechanisms of action. We also found that increasing polygenic risk for BD was associated with lower risk of treatment failure, at least over an initial stabilization phase of treatment. Despite using one of the largest available samples of post-mortem brains from the Lieber collection to generate more powerful gene expression prediction models, our study still suffers from lack of statistical power with sample size limitations from both the reference sample and the test sample along with other limitations of the PGBD discussed above.

The particular significance of the thesis work is that, although we retrospectively assessed the historical data on prescribing trends, we prospectively approached clinical and genetic evaluation of lithium response, which is a rarity in previous studies of lithium. The hope is that we will be able to improve upon our prior research efforts by developing more sophisticated models that incorporate both clinical and biological markers to predict treatment response. Given the devastating burden of BD, there is considerable motivation to develop more effective treatment options. The current pandemic adds to the environmental factors that will inevitably contribute to more mood dysregulation, which may eventually lead to development of the disorder. Lithium is an inexpensive and effective treatment, but it does not work for everyone. If validated, the findings from the thesis work may generate clues about the underlying mechanisms that explain differential lithium response in treating BD and help complete the clinical picture and genetic architecture for different patient profiles that respond differently to lithium so as to identify

them before starting treatment. By doing so, my thesis contributes to the ultimate goal of precision medicine for improving the treatment of patients with bipolar disorder.

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EDUCATION

Johns Hopkins Bloomberg School of Public Health

September 2016 – present

- PhD candidate in mental health (GPA: 3.91/4.0)
- Master's student in biostatistics (GPA: 3.91/4.0)
 - o Coursework: Statistical Machine Learning; Methods in Biostatistics; Statistical Methods in Public Health; Epidemiologic Methods; Statistics for Psychosocial Research; Analytic Strategies in the Genetics of Complex Diseases; Practice of Statistical Consulting, etc.
- Johns Hopkins Mental Health Scholar (highest departmental scholarship)
- Johns Hopkins 2017-2018 and 2018-2019 Centennial Scholar (top 0.4%)
- Johns Hopkins 2018-2019 Lucy Shum Memorial Scholarship

Fudan University

September 2012 – July 2016

- B.S. biological sciences (GPA: 3.28/4.0, National Top Talent Undergraduate Training Program, top 11.5%)

RESEARCH EXPERIENCE

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

August 2016 – present

- Identification of multi-omics biomarkers for cortisol levels and emotional stress (1 submitted, 1 working paper)
 - o Employed logistic regression and random forest to predict outcomes
 - o Identified differentially methylated regions and CpGs and differentially expressed genes through Methyl-Seq and RNA-Seq data
- Prediction of lithium treatment response and relapse for patients with bipolar disorder in the clinical trial - Pharmacogenomics of Bipolar Disorder (PGBD) (1 working paper)
 - o Employed Cox Proportional-Hazards model to model outcomes on patients on lithium monotherapy
 - o Built cross-validated prediction models using clinical and polygenic risk scores predictors
- Development of a transcriptome-wide-association-based survival analysis framework (1 working paper)
 - o Employed LASSO, elastic net, and Best Linear Unbiased Prediction (BLUP) to build brain-region-specific gene expression imputation models with cis-SNPs
 - o Identified suggestively predictive genes for differential relapse for patients in the PGBD trial

Johns Hopkins School of Medicine, Baltimore, MD

September 2014 – December 2014

- Analysis of Alzheimer's disease's pathological characteristics using the ImageJ software – acknowledged in a Nature Communications publication (doi: 10.1038/ncomms12082.)

PROFESSIONAL EXPERIENCE

Booz Allen Hamilton, Health Analyst Intern, Rockville, MD

August 2019 – October 2019

- Led the development of a cancer target identification toolkit that integrates various biomedical data sources
 - o Finished the RNA-seq data preprocessing and differential expression analysis pipelines for samples of acute myeloid leukemia, neuroblastoma, and mature B-cell lymphoma
 - o Identified potential cancer subtypes through unsupervised learning approaches including PCA, t-SNE and hierarchical clustering
 - o Inferred disease-associated cellular pathways by pathway centrality analysis
 - o Identified potential repurposed drug candidates with inferred pathways and druggable targets API

Johns Hopkins Graduate Consulting Club, Pro Bono Consultant, Baltimore, MD

April 2018 – June 2018

- Worked on a location-based analysis regarding philanthropic allocations on an 8-people team

LEADERSHIP

Mental Health Graduate Student Network, Co-founder, Baltimore, MD

April 2018 – present

- Co-created the network which connects 2,650 students to on and off campus resources
- Co-hosted National Alliance of Mental Illness' #IWILLLISTEN campaign and led the social media campaign

Johns Hopkins Department of Mental Health, PhD Mentor, Baltimore, MD

August 2018 – May 2019

- Mentored 4 master's students and provided advising hours on research opportunities and applications

Secretariat General, Student Union of the Department of Life Sciences, Shanghai, China

April 2013 – April 2014

- Wrote a union proposal and an end-year summary; organized the Weekly Executive Board Meetings

PROGRAMMING SKILLS

High performance computing with shell scripting

Data manipulation, analysis, and visualization with R (tidyverse and ggplot2), Stata, SAS, and SQL

Statistical modeling and machine learning with R

PUBLICATIONS

Zandi PP, Wilcox HC, Lin Y, et al. Genes as a Source of Risk for Mental Disorders. In Public mental health. Second edition. ed. Oxford ; New York: Oxford University Press; 2019.

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